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ASPHYXIA NEONATORUM AND THE VERNIX MEMBRANE

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A SIGNIFICANT number of asphyxial neonatal deaths are believed to be caused by vernix caseosa plugging the bronchioles and lining the alveolar ducts and walls. The presence of a vernix membrane may represent only a more serious manifestation of the aspiration of amniotic contents, but since no proof exists as to its causation, it may be discussed as a distinct entity. Although this condition has been frequently recognized by many investigators who have been concerned with infant mortality and experimental physiology, too little emphasis has been placed on it as a primary cause of death in liveborn infants.

Observers reporting on the vernix membrane in current literature and textbooks persist in discussing it in connection with pneumonia of the newborn and the stillborn. The membrane was first described by Johnson and Meyer[1] in 1925. During a study of pneumonia they encountered several newborn infants with a hyaline membrane coating the walls of the smaller respiratory passages. This membrane stained intensely with eosin, contained many fat droplets and gave a negative result when stained for fibrin. They believed that it was derived from dissolution of the epidermal cells and fat of vernix caseosa, which was converted into a viscous layer. The presence of the membrane was associated with pneumonia in some but not in all instances. The membrane was never observed in stillborn infants. Therefore it was believed that the membrane was formed only after respiration had been established and that intrauterine aspiration of amniotic fluid was a necessary antecedent. Johnson and Meyer therefore concluded that intrauterine aspiration of fluid must follow respiration established in utero as a result of some factor producing anoxia. Farber and Sweet[2] also expressed the belief that the intrapulmonary presence of large amounts of amniotic contents was an indication of intrauterine aspiration following intrauterine anoxia. In a presentation of 178 cases in which amniotic contents had been found in the lung, they

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1. Johnson, W. C., and Meyer, J. R.: Am. J. Obst. & Gynec. **9**:151, 1925.
2. Farber, S., and Sweet, L. K.: Am. J. Dis. Child. **42**:1372, 1931.

reported three neonatal deaths associated with vernix membrane in infants delivered by postmortem cesarian section. Russ and Strong [3] found that the infant mortality rate following cesarian section was 9 to 10 per cent when inadequate intratracheal aspiration was performed and less than 2 per cent when aspiration was done. Infants not treated with proper aspiration lived from four to forty-seven hours and died with symptoms of obstruction of the respiratory passages. In each one a "pseudo-membrane" was observed in the alveoli of the lungs, and they considered that this condition was a true aspiration pneumonia. Helwig,[4] in a report of 66 cases of pneumonia, mentioned finding a vernix membrane in the lungs in 2. Rosenthal [5] attributed the membrane to degenerative changes in the epithelial lining of the respiratory passages due to intrauterine anoxemia, a "desquamative anaerosis." Benner [6] said that in some cases in which there has been aspiration of large amounts of amniotic contents "there is evidence that respiration has been attempted, as the vernix appears to have been forced against the alveolar walls, where it lies as a membrane and forms a barrier to gaseous exchange. This membrane must therefore be recognized as a cause of asphyxia and early death of the infant." Macgregor [7] found 11 infants with hyaline membrane in a series of 541 consecutive necropsies on the newborn and expressed the belief that it resulted from aspiration of amniotic contents in utero following intrauterine anoxemia. In all cases pulmonary atelectasis was more severe than usual. Ample evidence of severe anoxia indicated that the infants had been asphyxiated at birth. She, too, stated that the vernix membrane often occurred without inflammatory reaction, which indicated that pneumonia was merely a complication and not the cause of the presence of the membrane. Labate [8] found the hyaline membrane in 2 per cent of fetal or neonatal deaths attributed to lesions of the lungs. He stated that from the point of view of etiology the condition has not been explained but that it may represent a late effect of intrauterine aspiration of amniotic fluid. The fluid may be absorbed and the solid elements converted into a hyalinized gummy substance. Potter [9] not only mentioned the hyaline membrane as a cause of neonatal death but thought that its presence should be suspected on a study of the clinical course of the newborn. Schenken [10] also wrote of this condition in his report of a series of deaths due to aspiration of amniotic contents.

3. Russ, J. D., and Strong, R. A.: Am. J. Obst. & Gynec. **51**:643, 1946
4. Helwig, F. C.: Am. J. Obst. & Gynec. **26**:849, 1933.
5. Rosenthal, M.: J. Pediat. **6**:71, 1935.
6. Benner, M. C.: Arch. Path. **29**:455, 1940.
7. Macgregor, A. R.: Arch. Dis. Childhood **14**:323, 1939.
8. Labate, J. S.: Am. J. Obst. & Gynec. **54**:188, 1947.
9. Potter, E. L.: Am. J. Clin. Path. **17**:524, 1947.
10. Schenken, J. R.: Nebraska M. J. **32**:362, 1947.

DESCRIPTION OF PATIENT AND MEMBRANE

Some of the infants who live for a period of a few hours to four days after birth exhibit fairly characteristic clinical signs of disease of the respiratory system prior to death. They may breathe normally or be slow to breathe at birth but eventually there develops an increasing struggle for breath. Dyspnea and cyanosis become manifest in spite of oxygen–carbon dioxide insufflation, and costal retraction may occasionally be present. At necropsy the changes are frequently limited to the lungs. The lungs of these infants are liver-like in consistence and sink when placed in water. Only a slight amount of aeration may be observed, along the anterior margins. Microscopically, a hyaline membrane is seen which stains with eosin. The membrane is plastered against the walls of opened alveoli, alveolar ducts and an occasional respiratory bronchiole. With sudan IV the membrane is stained red, both diffusely and in many small droplets. Toward the luminal side of the membrane one can occasionally see a basophilic stringy substance which gives a negative reaction for mucin. A variable number of epidermal cells may be found in this location. Vernix caseosa of exceptionally high lipid content and containing many epidermal cells is seen plugging the terminal bronchioles where these join the alveolar ducts. The alveolar ducts and alveoli which are lined with the membrane remain open. Most of the remainder of the lung is collapsed, a picture of atelectasis due to obstruction or to resorption. These changes may be associated with pneumonia in some instances. The occasional petechiae appearing on serous surfaces and in the brain are evidence of asphyxial death.

In contrast to the condition described, the lungs of other newborn infants who have died exhibit a different type of hyaline membrane. This type contains little or no lipid and is most frequently associated with interstitial pneumonia. In the lungs of these infants there are variable amounts of vernix caseosa and plugs of vernix do not appear to occlude the respiratory passages.

MATERIAL STUDIED

A study was made in two series of 119 consecutive necropsies on infants dying during the period 1938 to 1947. Liveborn infants of both series were all in the neonatal period, i. e., less than thirty days after birth. Permissions to make necropsies were obtained by members of the obstetric and pediatric staffs of the University Hospital.

The original series consisted of necropsies performed on 35 liveborn and 14 stillborn infants during 1946 and 1947. They were performed by the permanent and resident staff of the department of pathology of the University of Georgia.

Hospital charts were carefully examined for contributory causes of death or causes not evident at postmortem examination. The routine hematoxylin-eosin method of staining was followed, with use of Harris' hematoxylin solution. When we suspected that a vernix membrane was present, frozen sections were stained with sudan IV by Herxheimer's method. The first few lungs in which

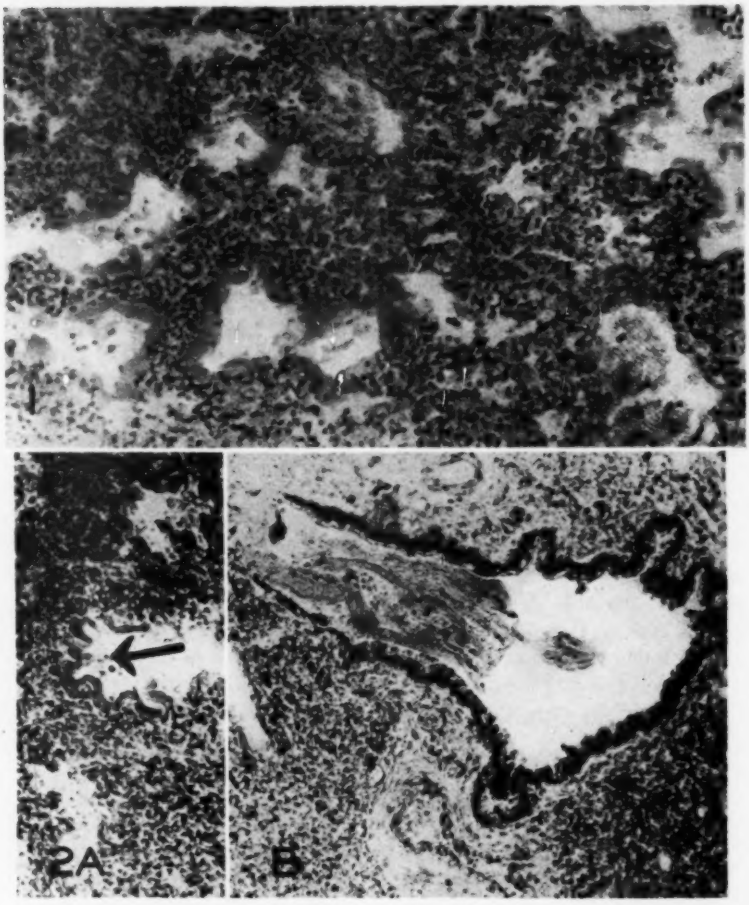

Fig. 1.—Membrane is seen lining alveolar ducts and sacs. Note extreme resorption atelectasis. Hematoxylin and eosin; low power.

Fig. 2.—*A*, lipid-rich vernix is seen lining a duct at the alveolar junction. Sudan IV. *B*, lipid-rich vernix is seen plugging a terminal bronchiole. Sudan IV; low power.

a vernix membrane was observed were also stained for mucin. In most instances sections were secured from each lobe of the lungs.

An intrapulmonary vernix membrane was demonstrated in 6 (12 per cent) of the 49 infants. Since the membrane does not occur in stillborn infants, this represents 17 per cent of all the liveborn infants on whom necropsies were made. In 2 instances only the vernix membrane was observed in the lungs. In addition to the membrane 2 infants had congenital pneumonia, and 2 died with an early postnatal pneumonia probably incident to resorption atelectasis of the lungs. There was no other evident cause of death in these infants.

CASE 1.—The infant was born at full term. Delivery was normal and spontaneous, with the mother under analgesia induced with intravenously injected scopolamine hydrobromide U. S. P. and pentobarbital sodium U. S. P. The membranes were intact until shortly before birth. Cyanosis was present at birth. The resuscitation procedure consisted of tracheal aspiration and administration of oxygen with the Torpin insufflator. Synthetic vitamin K (synkamin®) was administered. Little improvement was noted following this therapy. No fever was observed. The infant lived two days.

Microscopic examination of the lungs revealed an extensive vernix membrane and congenital pneumonia.

CASE 2.—The infant was born at full term. Delivery was normal and spontaneous with the mother under scopolamine and pentobarbital analgesia. The membranes ruptured at the onset of labor. The infant breathed normally at birth. A few hours later it was cyanotic and was exhibiting a grunting type of respiration. Intratracheal aspiration was performed and oxygen given by means of the Torpin insufflator. The infant improved somewhat, then became more cyanotic. No fever was noted. The infant lived one day.

Microscopic examination showed the lungs extensively involved with a vernix membrane.

CASE 3.—The infant was born at full term. Labor was long, and the infant was delivered by low forceps. Respiratory difficulty was noted shortly after birth and breathing became increasingly more labored. The baby became cyanotic and was placed in an oxygen tent. No fever was noted. The infant lived four days.

Microscopic examination showed the lungs moderately involved with a vernix membrane and early pneumonitis.

CASE 4.—The infant was born at full term. The mother was given "twilight sleep" and posterior pituitary injection U. S. P. The membranes were intact until the onset of labor. Breathing was spontaneous, and the infant had good color at birth. Cyanosis developed a short time after birth. Oxygen was given and the color improved. The oxygen gave out, and the baby became cyanotic again. It improved for only a short time when more oxygen was given. No fever was noted. The infant lived twenty-six hours.

Microscopically, the lungs showed an extensive vernix membrane and congenital pneumonia.

CASE 5.—The infant was born at full term. The history was incomplete. Respirations were poor at birth. Cyanosis was either present at birth or developed later. The temperature was 96.0 F. Oxygen was given. The infant lived eighteen hours.

Microscopically, the lungs were extensively involved with a vernix membrane.

CASE 6.—The infant was born at full term. Labor was of five hours' duration. Scopolamine hydrobromide and pentobarbital sodium were given. Delivery followed tetanic spasm of the uterus and was precipitate. The infant was pale, emaciated

and pulseless at birth. It later became cyanotic and had grunting respiration, which was deep and pauseless. Intratracheal insufflation of oxygen was given with a Torpin insufflator. The temperature became elevated. The infant lived four days.

Microscopic examination showed the lungs moderately involved with a vernix membrane and early pneumonitis.

The second series studied consisted of 70 consecutive necropsies, the material of which was obtained from the files of this department. It included 45 stillborn and 25 liveborn infants for the period 1938 to 1945 inclusive. One liveborn infant with multiple congenital abnormalities had pulmonary findings consistent with vernix membrane involvement and congenital pneumonia. No sections stained for fat were available. This was 4 per cent of all liveborn infants or 1.4 per cent of all infants, liveborn and stillborn, on whom necropsies were made.

The total number with vernix membrane in the two series therefore was 7 (11.6 per cent) of liveborn infants or 5.8 per cent of the total number of newborn infants on whom necropsies were made. Although the total number of cases of vernix membrane involvement in this series is small for comparison, the incidence is much higher than that reported by Macgregor [7] and Labate.[8]

COMMENT

A discussion of asphyxia neonatorum necessitates a short résumé of the perplexing problem of fetal respiration and is essential in attempting to show its etiologic relationship to the vernix membrane. Much work has been done on this subject in both human beings and lower animals with varying results. Windle [11] stated that under normal conditions the oxygenation of the fetus is adequate until shortly before term, when there is a progressive decline in placental efficiency. Only at this time do minor rhythms of respiratory movements occur. Normally the fetal musculature is atonic, and therefore it is incapable of initiating sufficient expansion of the lung for aspiration of amniotic contents unless the respiratory or other centers of the central nervous system are excited by a depression of thresholds or an elevation of carbon dioxide in the blood. Should oxygen saturation decrease markedly, numerous motor neurons are activated, muscle tonus increases and dyspneic gasping movements ensue. Surprisingly little activity has been noted in utero in lower animals by Windle. Farber and Sweet [2] expressed the belief that obstruction of placental circulation with resultant intrauterine anoxia initiates premature respiratory movements in utero with aspiration of amniotic contents. Oxidation

11. Windle, W. F.: Physiology of the Fetus, Philadelphia, W. B. Saunders Company, 1940.

is thereby further suppressed by obstruction of alveoli and bronchioles. Davis and Potter,[12] using the method of Dieckmann and Davis, withdrew amniotic fluid and injected colloidal thorium dioxide into the human amniotc sac. This was done on one series of patients during the first half of gestation and in another series at or near term. Local anesthesia was used, and pregnancy was terminated by elective cesarian section thirty minutes to fifty-two hours after injection. "All infants were alive and normal at birth." In most instances, after death of the fetus, thorium dioxide was found in the lungs by roentgenologic or microscopic examination. In these patients the authors noted spasmodic and irregular respiratory movements of the fetus in utero even in early fetal life. They believed that thorium was concentrated in the lungs as a result of fluid absorption by prealveolar and alveolar capillaries. Much amniotic fluid may escape in this manner, leaving thorium behind. Some of the fluid aspirated into the respiratory tract may escape back into the amniotic cavity by a tidal flow [13] normally present. Potter and Davis concluded that an aquatic existence of the fetus is normal during intrauterine life and that intrauterine respiratory activity is instituted in early pregnancy. Although spasmodic, irregular and shallow, it differs only slightly in pattern from extrauterine respiration, the major change being substitution of air for fluid.

In the present series varying amounts of amniotic contents were found in the lungs of infants known to have experienced intrauterine anoxia or hypoxia. These necropsies performed on both stillborn and liveborn infants included cases of premature separation of the placenta, prolapse of the cord and erythroblastosis fetalis. A vernix membrane was not demonstrated in any of these infants. Damage to the nervous system from anoxia probably accounts for the death of these infants. Therefore, although intrauterine anoxia may lead to exaggerated respiratory movements, this should not be considered the dominant factor responsible for development of the vernix membrane or the presence of much vernix debris, because many newborn infants are subject to varying degrees of intrauterine anoxia and a membrane can be demonstrated in only a few.

Factors other than simple aspiration of contents must be involved whether aspiration occurs normally or abnormally. Several of these factors must be considered. Since the sebaceous glands of certain persons are more active than others, one may assume that this is also true of the fetuses. Desquamation and dissolution of epidermal cells also are probably variable. It is known from clinical observation that the amount of vernix caseosa and amniotic debris in the fluid varies and

12. Davis, M. E., and Potter, E. L.: J. A. M. A. **131**:1194, 1946.

13. Snyder, F. F., and Rosenfeld, M.: Am. J. Obst. & Gynec. **36**:363, 1938.

that the proportion of amniotic debris under ordinary circumstances would vary inversely with amount of fluid. In addition, perhaps vernix may be concentrated not only in the amniotic sac but also in the respiratory passages, since it has been suggested by Davis and Potter [12] that there may be an exchange of amniotic fluid within the lungs by way of alveolar and prealveolar capillaries. If this is true, then vernix caseosa may be excessively concentrated or left behind because of absorption of fluid. Theoretically, then, in the presence of large amounts of concentrated vernix extremely high in lipids, conditions are ripe for development of the vernix membrane.

An attempt was made to produce a membrane in the alveoli of several lungs removed from stillborn infants at autopsy. A sample of amniotic fluid was centrifuged; 5 to 10 cc. of the sediment, warmed to body temperature, was injected into a similarly heated lung and alternating negative and positive pressure at 10 mm. of mercury applied in a glass chamber until the lung was expanded. In one lung a pseudomembrane formed in some of the respiratory bronchioles, but this membrane contained only small amounts of fat. None was seen in the alveoli. Although constant body temperature was not maintained during the entire procedure, it seems probable that the fluid was not sufficiently high in lipids to produce the characteristic picture. Furthermore, in these lungs there could be no exchange of fluids through capillary walls. Although a negative result was obtained in these instances, it seems to lend support to the foregoing theory. Further work is necessary on this phase of the problem.

In this seres there were 13 infants with so-called congenital pneumonia, 7 being stillborn infants. In 3 (50 per cent) of the 6 liveborn infants, the pneumonia was associated with a vernix membrane. Although it is believed by some that the association of pneumonia with the membrane is purely coincidental, the high occurrence of pneumonia in the infants exhibiting the vernix membrane is rather striking. Perhaps the presence of pneumonia causes abnormal stickiness of the cells lining the respiratory passages, such as occurs in endothelium during the phenomenon of inflammation, thereby permittng greater adhesive surfaces on which vernix may be deposited.

In the present series 2 of 5 infants with extrauterine pneumonia lived four days. These exhibited moderate involvement with the vernix membrane. It is well known that atelectasis predisposes to pneumonia; therefore, in these cases the membrane seems to be a definite predisposing factor.

Although Farber and Sweet [2] expressed the belief that there is no reaction in the lungs to amniotic contents and Johnson and Meyer [1] stated that a slight reaction may occur, there remain those patients

who might live with moderate amounts of vernix in the form of a membrane. Nelson and Venable,[14] however, reported a case in which a patient with symptoms at birth suggestive of asphyxial membrane involvement died at 14 months with acute bacterial endocarditis. The lungs at necropsy showed many alveoli obliterated by fibrous connective tissue and the walls of functioning ones thickened, "probably a result of amniotic fluid pneumonia." Of course, it is impossible to state that this "pneumonia" was in reality one of the vernix membrane type, but it is interesting to speculate on that possibility in this case.

Finally, what is the relationship between modern methods of resuscitation and development of the membrane? We have seen that, following cesarian section, if adequate immediate aspiration is done, the mortality rate may be lowered from 10 to 2 per cent. This lower mortality rate was apparently, for the most part, in a "pseudo-membrane" group of cases. This suggests that immediate intratracheal aspiration may, in some cases, prevent aspiration of excessive amounts of amniotic debris and more extensive membrane involvement.

It was thought that active resuscitation under normal pressures with the Torpin insufflator[15] might have increased the incidence of the membrane in the 1946-1947 series. However, the insufflator has been in use during the period of this entire series. Furthermore, insufflation is done on most stillborn infants and no membrane has yet been encountered. Therefore there is a discrepancy in the percentage with membrane involvement in the two groups which is unaccounted for because of this factor.[16] During the past two years infants have been placed in the bassinet with head elevated instead of depressed. It is thought this position may have led to poor drainage of the respiratory tract in cases examined during that period.

SUMMARY AND CONCLUSIONS

A vernix membrane forms in the lungs of a significant number of liveborn infants, which leads to asphyxia within a few hours to four days after birth. Although a high percentage also have pneumonia, there is usually no other obvious cause of death.

This membrane prevents exchange of gases in opened alveoli and is associated with vernix in terminal brochioles and alveolar ducts which produces atelectasis due to obstruction and resorption.

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14. Nelson, R. L., and Venable, D. R.: J. Lab. & Clin. Med. **26**:772, 1941.

15. Volpitto, P. P., and Torpin, R.: South. M. J. **35**:559, 1942.

16. Since this present series was completed a newborn infant has been encountered who never breathed spontaneously. Artificial respiration was given for four hours by means of the Torpin insufflator, and the heart continued to beat for that period of time. An intrapulmonary vernix membrane was found in this infant and was associated with pneumonia.

In these infants the vernix is extremely high in lipids and is present in large amounts in the respiratory tract and alveoli before birth.

In these infants the vernix may have been concentrated in the lung either because of absorption of fluid by way of exchange through alveolar and prealveolar capillaries or because only small amounts of amniotic fluid were present.

The presence of the membrane may represent only a variant of the aspiration of amniotic debris and vernix caseosa so frequently found to be a cause of asphyxia neonatorum.

Following the first extrauterine breath, this material in part is forced against the walls of alveoli and alveolar ducts, while that present in the upper respiratory tract lodges in the respiratory bronchioles. As extrauterine respirations become more forceful, greater expansion takes place, and more vernix is aspirated into the smaller units from above.

Its high incidence in association with congenital pneumonia is probably due to the increased stickiness of the respiratory passages which has developed during the inflammatory process.

Intrauterine anoxia may produce exaggerated intrauterine respiratory movements with aspiration of greater amounts of amniotic fluid, but death in that case is primarily due to damage within the central nervous system.

It is postulated that the necessary factor concerned with development of the membrane is concentration of vernix which has an extremely high lipid content. Pneumonia prepares a fertile field for its development. Exaggeration of intrauterine respiratory movements is a factor only in those cases presenting the aforementioned prerequisites.

Aspiration may be of no avail in many cases when much of the vernix is beyond the tracheal bifurcation, but in others it may forestall more extensive involvement of the lung, and death of the infant may be prevented.

IN VITRO STUDIES OF LYMPH NODES INVOLVED IN HODGKIN'S DISEASE

I. Liquefaction of Culture Medium

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TISSUE cultures of lymph nodes involved in Hodgkin's disease develop according to a pattern peculiar to, but not specific for, this disease. The pattern consists of the following three easily demonstrated and frequently recurring phenomena: (1) evolution of giant cells; (2) intracytoplasmic inclusions; (3) liquefaction of areas of the culture medium. We have studied all three of these phenomena and wish at this time to present our analysis of the liquefaction of the medium.

HISTORICAL REVIEW

In 1921 Lewis and Webster [1] reported their study of cultures of human lymph nodes grown in vitro. They noted that liquefaction occurred on the first and second days after implantation but made no further comment on it. A year later Maximow [2] stated that in cultures of rabbit lymph nodes the liquefaction of fibrin commonly observed in cultures of epithelium did not take place. The first comprehensive report of in vitro studies of Hodgkin's disease was made by Mankin,[3] in 1936. He observed that liquefaction did not occur in cultures of tuberculous nodes and did not occur, as a rule, in cultures of leukemic nodes but that it was prominent in cultures of lymph nodes involved in Hodgkin's disease and that it usually began on the sixth or the seventh day. In 1937 Meier [4] presented his findings in cultures of nodes affected by Hodgkin's disease but made no mention of liquefaction. Like Mankin, Grand [5] observed liquefaction during the course

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1. Lewis, W. H., and Webster, L. T.: J. Exper. Med. **33**:261, 1921.

2. Maximow, A.: Arch. f. micr. Anat. **96**:494, 1922.

3. Mankin, Z. W.: Beitr. z. path. Anat. u. z. allg. Path. **96**:248, 1936.

4. Meier, R.; Posern, E., and Weizmann, G.: Virchows Arch. f. path. Anat. **299**:329, 1937.

5. Grand: Personal communication to the authors.

of his studies of tissue cultures of lymph nodes from patients with Hodgkin's disease. The primary concern of his preliminary studies was the intracytoplasmic inclusions and the possibility that these were related to virus activity, but in later studies he concentrated on liquefaction and the possibility that it was related to an etiologic agent. He demonstrated that liquefaction of medium could be induced by adding drops of extracts of the diseased lymph nodes to cultures of chick chorioallantois.[5] During the period 1945 to 1947 Hoster[6] noted liquefaction when normal guinea pig spleen was grown in medium to which cell-free extracts and sediments of diseased tissue had been added; liquefaction also occurred in the controls, but much less often.

In our study we were chiefly interested in (1) the specificity of the liquefaction, (2) the degree of liquefaction produced by fragments of nodes in different histologic stages of Hodgkin's disease, (3) the degree to which liquefaction is related to the clinical picture, (4) the dynamics and the mechanism involved in producing the phenomenon.

MATERIAL AND METHOD OF STUDY

Fifty-six lymph nodes were studied, 29 from 26 patients with Hodgkin's disease (table 1) and 27 from 27 patients who did not have Hodgkin's disease (table 2). Two of the patients with Hodgkin's disease had two biopsies each and 1 patient had three biopsies, all being performed at six to twelve month intervals. After removal the lymph nodes were placed in Ringer's solution and cultivated within one-half hour. Each node was first rinsed with a solution of penicillin (500 units per cubic centimeter in Tyrode's solution) and then sectioned. One portion was kept for histologic study; the other, for tissue culture. After thorough washing with Tyrode's solution, small fragments were cut from representative portions of the node and cultivated according to Maximow's double cover slip method. The medium consisted of 1 volume of adult chicken plasma, 3 volumes of human placental cord serum and 1 volume of chick embryo extract. Two or 3 drops of this mixture were sufficient to make a clot on the small cover slip. Usually two fragments were embedded in each clot and incubated at 37.5 C. Every two or three days the cultures were washed in warm Tyrode's solution for twenty minutes, and one drop of the original medium was placed on the surface of the clot. From 20 to 60 implanted pieces of tissue from one node constituted a series. At twenty-four hour intervals, two or more slides were fixed in Carnoy's solution, Zenker's formol or Ringer's formol.[6a] As a rule, the experiment was terminated at the end of seven days, since we had found by experience that in this interval of time most of the explants and their outgrowths had been replaced by fibroblasts.

RESULTS

Usually by the second or the third day a ringlike area, or zone, of liquefaction was found at one side of the explant. Mankin called this a "liquefaction vacuole," and we, also, shall so designate it (fig. 1). When first observable the phenomenon suggests that a thin margin is separating from the periphery of the explant

6. Hoster: Personal communication to the authors.

6a. R. C. Parker: Methods of Tissue Culture, New York, Rockefeller Institute, p. 171.

(fig. 2). This margin becomes displaced for a variable distance beyond the parent explant, but, as a rule, the explant and the displaced margin remain connected at two opposite points (fig. 1, *E-E*); thus there is formed a boundary about a semilunar or roughly spherical area in which the medium, previously solid, has become a fluid in which the cell population can be seen floating about. This population must be differentiated from the cells floating in fluid expressed by the clot during gelation. If the culture is washed at this time, these cells float away, and a clear area is left behind. The displaced marginal fragment is composed of cell elements the same as those present at the intact surface of the explant, namely, lymphocytes, reticulum cells and also fibroblasts, provided displacement has taken

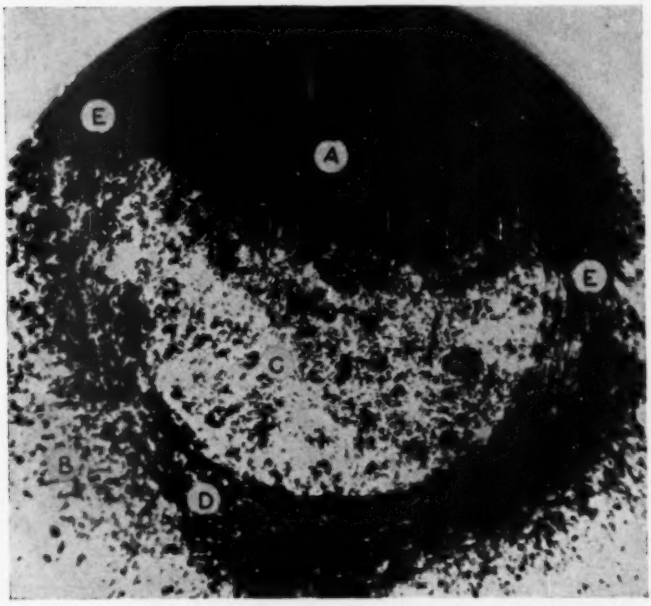

Fig. 1.—Photomicrograph of a fragment of a lymph node involved in Hodgkin's disease in which a liquefaction vacuole is fully formed. *A*, is the tissue fragment; *B*, the outgrowth or zone of migration; *C*, the area of liquefaction; *D*, the displaced margin of the fragment; *E-E*, the points at which the displaced margin of the fragment maintains continuity with the parent tissue.

place subsequently to the time at which the fibroblast-like cells develop in the explant (usually on the third or the fourth day). The displaced margin appears to rest on solid, unchanged medium. Within the liquefaction vacuole there is a mixed population made up usually of reticulum cells, lymphocytes and multinuclear giant cells, though these constituents may vary in different series, on different slides and in two explants on the same slide. Also within the liquefaction vacuole there is considerable activity, such as immigration of new cells coming from the explant, cell multiplication, cell degeneration and phagocytosis. In those cases in which the

vacuole forms before fibroblastic proliferation has begun, the fibroblasts, after their advent, begin to migrate from the explant and, creeping along the border of solid medium, they completely encircle this at its periphery. They continue to proliferate, arrange themselves so as to form an ever-thickening capsule and then invade the area of liquefaction (fig. 3). By the seventh day or later this area has become completely filled and is indistinguishable from areas not previously liquefied. These so-called liquefaction vacuoles vary in size, shape and number. As a rule, each explant develops but one; occasionally as many as three are found.

Having noted the dynamic histologic pattern of the liquefaction, we next concentrated attention on the specificity and the constancy of the liquefaction

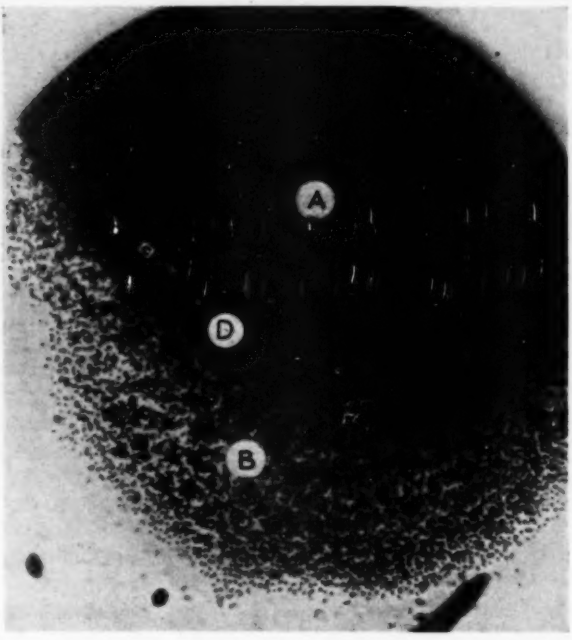

Fig. 2.—Photomicrograph showing an early stage of the formation of the liquefaction vacuole, which at this time is a narrow slit (*C*). The other letters in this and other figures to follow are explained in the legend for figure 1.

produced by the diseased nodes when these were grown in vitro. Soon it became apparent that neither was the liquefaction specific for Hodgkin's disease (fig. 4), nor did it occur constantly (table 1). Nevertheless, it was produced much more often by the nodes involved in Hodgkin's disease than by nodes not involved in this disease, grown under identical conditions (tables 1 and 2). Tables 1 and 2 show that 62 per cent of Hodgkin's disease cultures underwent liquefaction of medium, compared with 33 per cent of the cultures of nodes involved in diseases other than Hodgkin's disease. Cultures of tuberculous nodes also had a high incidence of liquefaction. In 5 cases of malignant lymphoma (2 of lymphocytic

TABLE 1.—*Data on Liquefaction of Medium of Cultures of Lymph Nodes Affected by Hodgkin's Disease*

Specimen	1-11	12	13	14	15	16	17	18	19	20	21*	22*	23†	24†	25†	26‡	27‡	28	29
Explants per series	11-54	56	22	7	22	47	44	35	38	21	18	13	37	49	67	37	29	1	2
Explants showing liquefaction	0	35	6	7	5	10	26	17	16	4	9	1	4	6	0	8	7	1	2
Percentage showing liquefaction	0	62	27	100	22	21	59	48	42	19	50	7	10	12	0	21	25	..	..
Day liquefaction appeared	..	1	2	5	3	3	2	2	4	3	4	7	2	2	0	2	3	..	..
Histologic diagnosis of disease of node	7 G 1 PG 3 S	G	G	G	GF	G	G	G	GF	G	PG	PG	G	G	G	G	G	PG	G

* Specimens 21 and 22 were taken from the same patient at two biopsies made at different times.
† Specimens 23, 24 and 25 were taken from the same patient at three biopsies made at different times.
‡ Specimens 26 and 27 were taken from the same patient at two biopsies made at different times.
G stands for the granulomatous form of Hodgkin's disease; PG, for the pregranulomatous form of Hodgkin's disease; S, for the sarcomatous form of Hodgkin's disease; GF, for the granulomatous form of Hodgkin's disease, fibrotic stage.

TABLE 2.—*Data on Liquefaction of Medium of Cultures of Lymph Nodes Affected by Diseases Other Than Hodgkin's Disease When Representative Fragments of the Nodes Were Grown in Vitro*

	Nonspecific Hyperplasia			Tuberculosis					Anthracosis			Lymphoma						Metastatic Carcinoma	
Specimen	1	2	3-11	12	13	14	15	16	17	18	19	20*	21†	22†	23‡	24†	25‡	26	27
Explants per series	38	36	270	50	28	36	30	40	20	22	22	48	48	7	40	64	40	11	60
Explants showing liquefaction	3	1	0	17	10	4	0	0	4	0	0	0	0	4	9	0	0	1	0
Percentage showing liquefaction	7	2	0	34	33	11	0	0	20	0	0	0	0	57	22	0	0	9	0
Day of appearance	5	3	0	3	5	4	0	0	3	0	0	0	0	3	2	0	0	..	0

* Specimen 20 was involved in lymphatic leukemia.
† Specimens 21, 22 and 24 showed reticulum cell sarcoma.
‡ Specimens 23 and 25 showed lymphosarcoma.

sarcoma, 3 of reticulum cell sarcoma) it occurred twice, or in 40 per cent. If the Hodgkin's disease group is compared with the non-Hodgkin's diease group with respect to the relative incidence of liquefaction, the following figures are obtained: 164 of the 445 Hodgkin's disease cultures, or 37 per cent, and 53 of the 226 non-

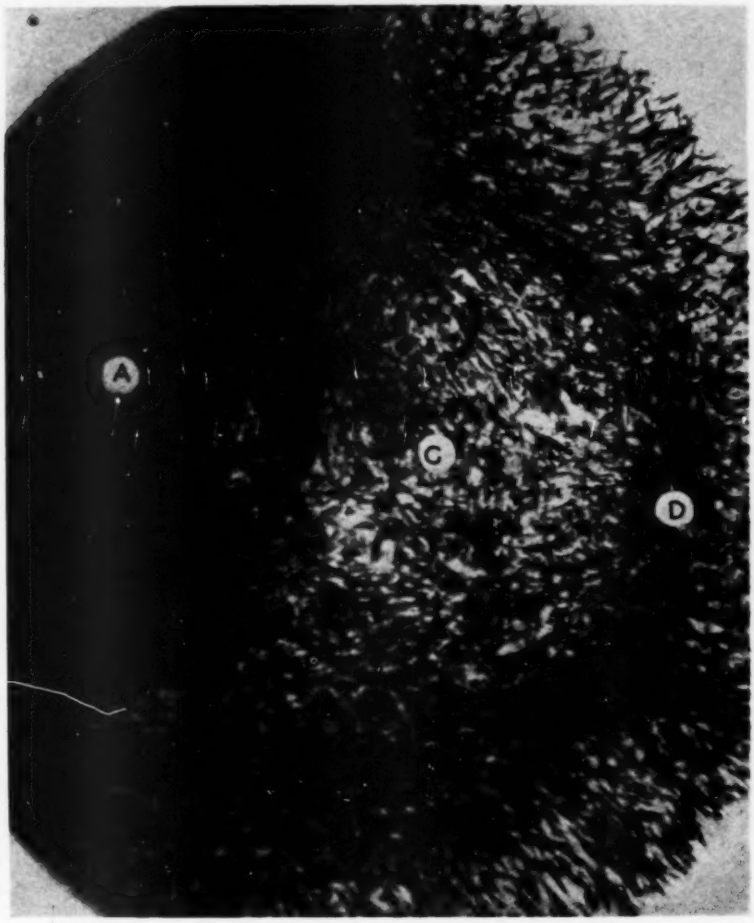

Fig. 3.—An area of liquefaction being overgrown with fibroblast-like cells. By the fourteenth day such areas may become completely filled.

Hodgkin's disease cultures, or 19 per cent, underwent liquefaction. In this calculation we did not include culture series, normal or diseased, showing no liquefaction whatever.

An attempt was next made to determine whether nodes with one histologic type of Hodgkin's disease produced liquefaction more often than nodes with a different histologic type. The only impressive finding was that cultures of the sarcomatous nodes failed to liquefy. All of the cultures of the more fibrous Hodgkin's disease nodes and about two thirds of those of the lymphocytic and of the granulomatous type did liquefy. No correlation could be established between this peculiar behavior of the node grown in vitro and the duration of disease, the tempo of disease or the clinical symptoms observed. In 3 instances multiple biopsies were performed. The third node removed from one patient failed to produce any cultures which liquefied; a second node from another patient gave a series which showed marked reduction of the number of cultures which liquefied—7 per cent, compared with 50 per cent of the cultures from the first node—and two nodes from a third patient produced an equal number of cultures in which liquefaction occurred.

COMMENT

When lymph nodes involved in Hodgkin's disease are grown in vitro under the conditions outlined, liquefaction of medium frequently occurs. This phenomenon, however, is neither peculiar to, nor specific for, Hodgkin's disease, for the same behavior is exhibited by cultures of lymph nodes affected by other diseases, namely, tuberculosis, lymphosarcoma, anthracosis, metastatic carcinoma and simple lymphoid hyperplasia. Despite this lack of specificity, however, the lymph nodes involved in Hodgkin's disease produce liquefaction with sufficient frequency to warrant ascribing significance to it, even though this significance is at present not understood. The fact that liquefaction occurs in tissue cultures of nodes affected by nonrelated diseases points to the existence of some common fundamental biologic mechanism latent in all nodes; that which renders the understanding of this mechanism perplexing is the extreme irregularity with which it is set into motion. Why, for instance, did only 1 of 37 fragments of a hyperplastic node exhibit liquefaction? Why did 35 of 56 fragments liquefy in one instance of Hodgkin's disease and only 5 of 22 in another? The most obvious way in which to begin an attempt to explain these facts seemed to be to relate this phenomenon, if possible, to the varying histologic aspects of the parent lymph nodes. When this had been done, no criterion emerged by which one could predict whether liquefaction would or would not take place in a given series of cultures. Comparison of the tissue culture behaviors of multiple nodes taken at different intervals from each of 3 different patients obscured the matter still further, since nodes from the same patient exhibited different behaviors even though the histologic aspects were similar.

Since the medium used in our tissue cultures was a fibrin coagulum, the obvious explanation of its liquefaction would be action by a fibrin-

olytic enzyme. A similar explanation was offered by Santesson,[7] who studied liquefaction occurring in cultures of epithelial tissue. We have not yet made a systematic study of enzymosis, but attempts to demonstrate proteolytic enzymes in lymphocytes have already been made by several workers. In 1908 Longcope and Donhauser [8] demonstrated that proteolytic enzymes were active, in acid medium, in the cells of a large cell lymphatic leukemia and absent in small lymphocytes; Morris and Boggs [9] (1909) extracted a protease from small lymphocytes obtained from a patient with chronic lymphatic leukemia and found the enzyme to be active in a neutral medium; Rona and Kleinmut [10] studied proteolytic properties of a lymph node and of spleen extracts and found kathepsin, active at p_H 4 to 5, and traces of trypsin, active at p_H 7 to 8; in rabbit lymphocytes Barnes [11] found small quantities of nuclease, amylase, lipase, lysozyme and adenosinase, and from cat lymphocytes he obtained kathepsin, nuclease, lipase and lysozyme. Suggestive preliminary studies, with tissue cultures as a test medium, have already been made by Grand.[5] He added extracts of lymph nodes affected by Hodgkin's disease in vitro to cultures of chick chorioallantois and obtained liquefaction of the medium. The same extracts added to medium alone, devoid of any implant, were said not to induce liquefaction. This would suggest that if enzymes were contained in the extracts they were inactive on the fibrin clot in the absence of living tissue, and this supposition seems supported by our studies, for in no instance did liquefaction occur when the fragment failed to grow.

Thus the *vis a tergo* for liquefaction of medium resides in the living cells of the fragment and is dependent for its operation on the active growth of these cells. In addition to this, there seems to be a second factor contributing to liquefaction—a factor which probably is related to conditions prevailing at certain regions of the periphery of the explant, since it always occurs there. Whether the activity begins immediately within or immediately outside the margin of the explant is difficult to determine, but at any rate some stretches of the periphery are favorable to reaction to an unknown stimulus and some are not. One of the favorable conditions may well be an optimum hydrogen ion concentration. This and other possible factors merit further study.

Also to be determined is which cell or cells growing in the culture are responsible for the elaboration of the liquefying factor—the lympho-

7. Santesson, L.: Acta path. et microbiol. Scandinav., 1935, supp. 24, p. 85.
8. Longcope, W. T., and Donhauser, J. L.: J. Exper. Med. **10**:618, 1908.
9. Morris, R. S., and Boggs, T. R.: Arch. Int. Med. **8**:806, 1911.
10. Rona, P., and Kleinmut, H.: Biochem. Ztschr. **241**:283, 1931.
11. Barnes, J. M.: Brit. J. Exper. Path. **21**:264, 1940.

cyte, the reticulum cell, the multinuclear giant cell or the fibroblast. In various cultures which came under our observation we frequently

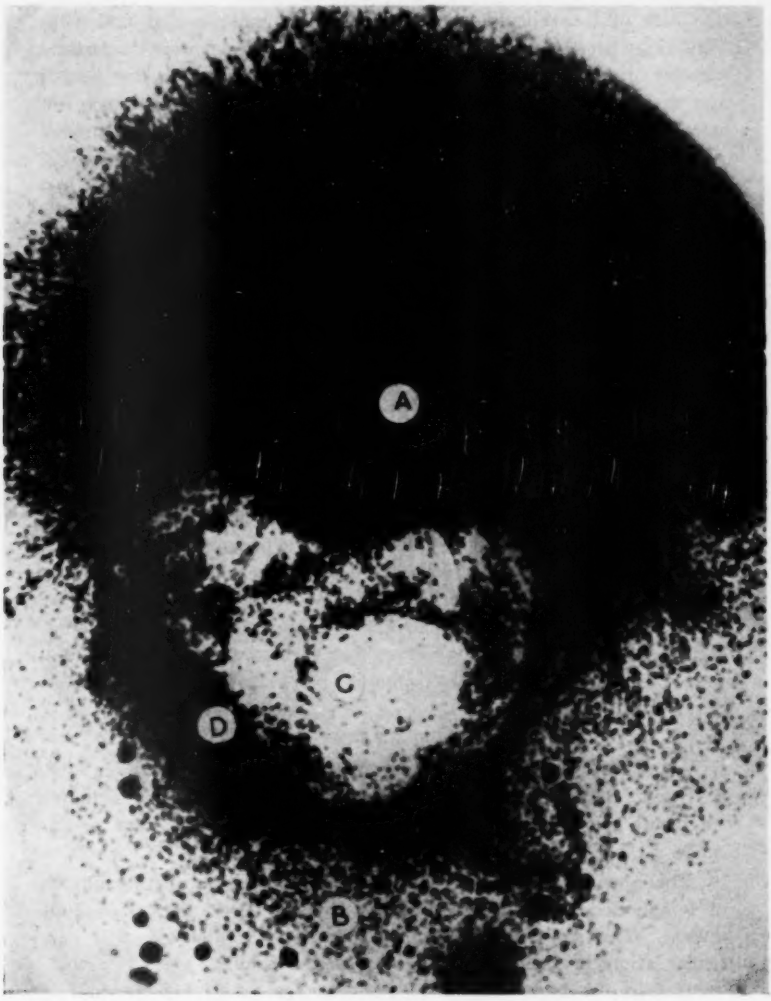

Fig. 4.—A liquefaction vacuole forming in a fragment of a tuberculous lymph node.

noted lymphocytes growing out from one side of the explant in dense, compact masses or diffusely from the entire periphery. Although

these underwent disintegration, no liquefaction occurred, or if it did occur, it appeared in areas other than those in which the disintegrating cells in question were present. The same observation was made in the case of the reticulum cells. This would indicate that under the conditions present neither of these types of cells elaborated a ferment capable of effecting lysis. One may not conclude from this that neither the reticulum cell nor the lymphocyte is capable of producing the enzyme, for in one series of cultures made from a lymphocytic lymphosarcoma, a sarcoma composed of small, mature lymphocytes, 9 of 22 cultures exhibited liquefaction, and in another series, made in a case of reticulum cell sarcoma, 4 of 7 implants liquefied. The fibroblast can be dismissed as a possible agent, since liquefaction never occurred late in the life of the culture, when fibroblastic proliferation was most vigorous. For a time we were inclined to ascribe the phenomenon to the multinuclear giant cells because of the fact that these were so conspicuously present in the liquefaction vacuoles. However, we found many zones of liquefaction free of giant cells and observed giant cells present in nonliquefied areas. The fact that formation of multinuclear giant cells was so frequently associated with liquefaction of medium suggests that some sort of interrelationship exists. Perhaps the factor which induces reticulum cells to transform into giant cells also induces them to elaborate fibrinolytic substances. Finally it should be added that even though liquefaction is a phenomenon nonspecific for Hodgkin's disease (as we believe our experiments have demonstrated) it must not therefore be assumed that liquefaction is always evidence that some one particular etiologic agent is at work. In pathologic tissues one frequently observes that varied and nonrelated stimuli induce similar reactions; the same may be true of tissue reactions observed in vitro.

SUMMARY

Liquefaction of medium occurs with sufficient frequency in cultures of lymph nodes involved in Hodgkin's disease, when representative fragments are grown in vitro, to warrant ascribing to it some significance.

The phenomenon is not specific, since it also occurs in cultures of lymph nodes that are affected by diseases other than Hodgkin's disease. This does not necessarily indicate the existence of a particular etiologic agent responsible for liquefaction.

It seems probable that liquefaction is due to an enzyme, but no biochemical studies have yet been undertaken to establish this as a fact.

It cannot be stated as a fact that the hypothetic ferment is induced by the lymphocytes or by the reticuloendothelial cells, singly or in combination, but it seems to us that the reticulum cell may possibly be the agent responsible.

We are of the opinion that certain favorable conditions must be created in order for liquefaction to take place and that these conditions occur at the periphery of living and actively growing explants.

There is no relationship between the occurrence and the degree of liquefaction of medium, on one hand, and the histologic aspects of the lymph node and the clinical aspects of Hodgkin's disease, on the other.

IN VITRO STUDIES OF LYMPH NODES INVOLVED IN HODGKIN'S DISEASE

II.. Tissue Culture Studies; Formation, Behavior and Significance of the Multinucleated Giant Cell

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IN A previous communication [1] it was stated that when grown in vitro lymph nodes affected by Hodgkin's disease displayed certain phenomena which, though nonspecific, were nonetheless striking and of possible significance. In the communication referred to, one of these phenomena, namely, liquefaction of medium, was discussed in detail. We now wish to describe and discuss the possible significance of a second phenomenon—the occurrence of multinucleated giant cells.

REVIEW OF THE LITERATURE

In tissue cultures of nodes involved in Hodgkin's disease, tuberculous nodes and nodes showing chronic nonspecific adenitis, Lewis and Webster [2] observed giant cells similar to those we shall describe. Several years later Lewis [3] reviewed the subject of the giant cells that form in tissue cultures, the frequency of their occurrence and the lack of relationship to any specific disease, and demonstrated that their appearance was limited neither to lymph nodes nor to any one species of animal. He observed multinucleated giant cells alike in cultures of spleen and of marrow from human beings, mice, rats, chickens, rabbits, guinea pigs, dogs, fish, amphibia and reptiles, and in cultures of cells of buffy coats prepared from blood specimens of this same group of animals. Regardless of whether cells for culture were obtained from buffy coat or from tissue fragments of spleen or lymph node, and regardless of the species of animal from which these were procured, the giant cells emerging from them in vitro appeared to be alike.

From the Hodgkin's Disease Research Laboratory, St. Vincent's Hospital.

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1. Rottino, A., and Hollender, A.: Arch. Path., this issue, p. 317.
2. Lewis, W. H., and Webster, L. T.: J. Exper. Med. **33**:261, 1921.
3. Lewis, W. H.: Am. Rev. Tuberc. **15**:616, 1927.

Mankin,[4] in his tissue culture of Hodgkin's disease nodes, also noted multinucleated giant cells, which made their appearance on or about the seventh day, gradually increased in numbers and then degenerated in eight to ten days. They sometimes attained large size and developed as many as 80 to 100 nuclei. It was Mankin's opinion that the multinucleated giant cells under discussion developed from reticular cells and bore no direct histogenic relationship to Sternberg-Reed cells. He noted too that the multinucleated giant cells occurred more often in cultures of Hodgkin's disease nodes than in cultures of nodes not affected by Hodgkin's disease.

Meier,[5] too, found giant cells conspicuous in tissue cultures prepared from Hodgkin's disease nodes. He failed, however, to differentiate between various types of giant cells and hence, when he refers to "giant cells" as possible Sternberg-Reed cells, confusion arises as to the precise cells to which he is referring.

Grand [6] denominated the type of multinucleated giant cell under discussion in the present paper as the Sternberg-Reed cell. Stout [7] agreed with this opinion and added that the cells began as typical Sternberg-Reed cells and that after migrating from the explant, they change their appearance and resemble thereafter foreign body giant cells.

Hoster [8] observed multinuclear giant cells in tissue cultures of normal uninoculated cells. He concluded that they were not specific but represented in some instances a reaction to a contaminant—chicken lymphoma virus—introduced into the culture medium via chicken embryo extract and chicken plasma obtained from flocks known to have a high incidence of lymphomatosis. In other instances the giant cells, according to Hoster, represent a reaction to cell-free Hodgkin's disease material added to normal cells in tissue culture.

Our interpretation has been at such variance with these later opinions that we feel it pertinent to present our views and the findings on which they are based.

MATERIAL

The material and the method used in the present study were outlined in our first paper.[1] Briefly, they were as follows: Twenty-eight lymph nodes from 28 persons who did not have Hodgkin's disease and 27 nodes from 23 patients with Hodgkin's disease were used. A series of cultures were made from each node, Maximow's double cover slip method being used. At twenty-four hour intervals

4. Mankin, C. W.: Beitr. z. path. Anat. u. z. allg. Path. **96**:248 and 308, 1936.

5. Meier, R.; Posern, E., and Weizmann, G.: Virchows Arch. f. path. Anat. **299**:329, 1937.

6. Grand, C. G.: (*a*) Proc. New York Path. Soc., 1945, p. 137; (*b*) Proc. Soc. Exper. Biol. & Med. **56**:229, 1944.

7. Stout, in discussion of Grand.[6a]

8. Hoster, H.: Personal communication to the author.

slides with their cultures were fixed in various fixatives and stained with Giemsa stain. That the multinucleated giant cell is of some significance in Hodgkin's disease was deduced from statistical evaluation of comparative observations of the Hodgkin's disease and non-Hodgkin's disease groups.

OBSERVATIONS

The giant cell under discussion is a large, multinucleated structure of varying size and shape. Some specimens are of enormous size (fig. 1), thin, spread out, with barely perceptible margins, while others are smaller, more oval, with visible borders (fig. 2). Conspicuous in the giant cell is the large number of nuclei—sometimes as many as 100 or more—arranged about a central hyperchromatic mass which, when decolorized, is seen to contain particulate matter, the particles being of various sizes. If the hyperchromatic mass is absent, there is a more diffuse distribution of the nuclei. Within the cytoplasm vacuoles containing ingested cells and cell fragments are frequently found.

The multinucleated giant cells are usually seen on the third and fourth days of the culture—earlier in some instances, later in others. They appear in the central fragment, in the zone of migration and in the liquefaction vacuole. New giant cells are subsequently observed until the tenth to fourteenth day, or later, depending in part on when the culture becomes overgrown with fibroblasts. The life span of giant cells varies; It may be as short as three days. While young, the cells are barely visible; within twenty-four hours they become more distinct and their cytoplasm becomes granular; from forty-eight hours on they shrink and finally disintegrate.

Typical Sternberg-Reed cells were indubitably identified in cultures of only a few Hodgkin's disease nodes. In these cultures they bore strong resemblance to the Sternberg-Reed cell seen in tissue sections of nodes removed at biopsy and hence they easily could be distinguished from the large multinucleated giant cells under discussion.

Having satisfied ourselves as to the histologic aspects of the giant cell and having identified it as morphologically different from the Sternberg-Reed cell, we next considered its specificity and attempted to find quantitative and qualitative differences in its behavior which would furnish culture criteria for differentiating Hodgkin's disease from other forms of adenitis and lymphoma.

It quickly became apparent that the giant cell in question is not peculiar to any specific disease affecting the node, since it occurred in cultures of hyperplastic nodes and tuberculous nodes as well as in cultures of Hodgkin's disease nodes and lymphoma of other types. Quantitative differences were, however, noted. In the Hodgkin's disease group, giant cells were found in cultures of more nodes and in more cultures from each node, and were present in greater numbers per fragment cultured, than was the case in the non–Hodgkin's disease group. Of 27 Hodgkin's disease nodes, 16 (59 per cent) produced giant cells as compared with 9 of 28 (32 per cent) of non-Hodgkin's disease nodes. Both nodes and fragments which produced multinuclear giant cells in tissue culture will be referred to hereafter as "positive." The 16 positive Hodgkin's disease nodes produced 180 positive fragments (31 per cent of the 590 planted). The 9 positive non–Hodgkin's disease nodes produced only 30 positive fragments (13 per cent of the 228 planted). The total number of giant cells in the positive fragments of Hodgkin's disease nodes was 4,349 against 335 in the non–Hodgkin's disease nodes. This meant that the average Hodgkin's disease positive fragment contained about 23 giant cells, contrasted with 11 giant cells contained by the non–Hodgkin's disease fragments.

Fig. 1.—Photomicrograph of a multinucleated giant cell observed in tissue culture of a fragment of a lymph node from a patient with Hodgkin's disease. The cell is large, spread out thin; the outline is indistinct; the cytoplasm, pale; the nuclei are numerous and disorderly in arrangement. Present in the cytoplasm are other smaller, rounder and more hyperchromatic nuclear masses representing remains of ingested cells, such as lymphocytes.

Close scrutiny of the giant cells revealed certain detectable cytologic differences between the two groups. For instance, in cultures of Hodgkin's disease nodes more of the giant cells were apt to be larger and to contain greater numbers of nuclei. No differences in phagocytosis could be detected; in both groups many multinucleated giant cells were actively phagocytic.

Attempts to correlate the clinical state of the patient with Hodgkin's disease and the duration and tempo of the disease with giant cell behavior proved unsuccessful.

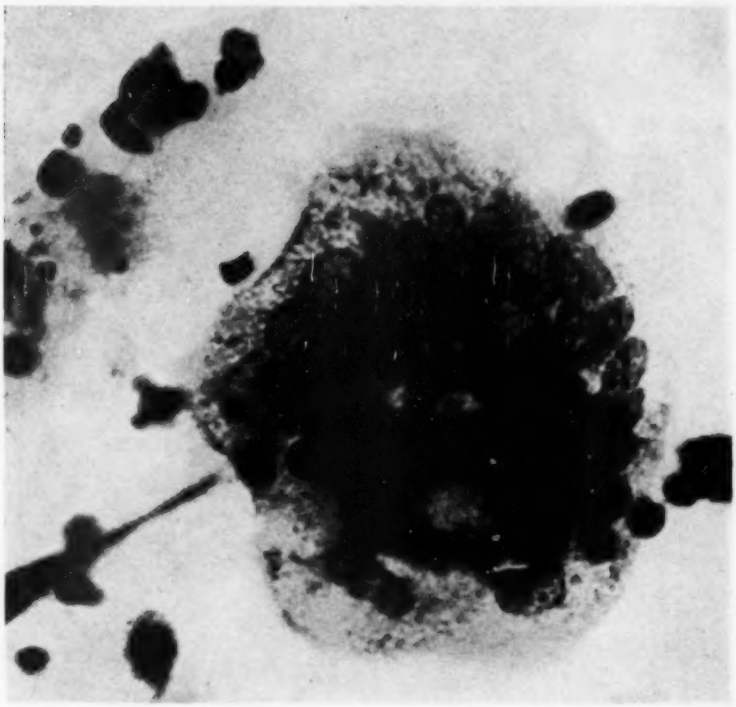

Fig. 2.—Another form of multinucleated giant cell from a preparation similar to that from which the giant cell of figure 1 was taken. The cell is smaller, more compact; the outline is more distinct; the nuclei are arranged about a central hyperchromatic mass. Both inside this mass and outside it dark, round nuclear remains of ingested cells are to be seen.

It was likewise impossible to discern any connection between the phenomenon of giant cell formation and behavior and the histologic picture of the nodes transplanted, except that in none of the three sarcomatous nodes were giant cells observable in culture. This was in contrast to fragments of other types of Hodgkin's disease nodes, such as the granuloma and the paragranuloma, from some of which giant cells emerged and from others of which they did not emerge.

Nodes from persons on whom several biopsies were done at different times showed diversity of behavior in vitro, even though the histologic aspects remained the same. Thus, in cultures from the first node of 1 patient there were few giant cells, and these few were small and had few nuclei; in cultures of the second node, however, giant cells were much more numerous, were larger and contained more nuclei. The same variation of behavior occurred between nodes of first and second biopsies of another patient; six months later, when cultures were prepared from a third biopsy specimen, no giant cells appeared.

COMMENT

The multinuclear giant cells observed in tissue cultures of lymph nodes involved in Hodgkin's disease do not appear to be Sternberg-Reed cells but rather cells of the foreign body type. They occur in vitro in cultures of tuberculous nodes, lymphosarcomatous nodes and nodes affected by nonspecific adenitis. They occur also in cultures of buffy coats of centrifuged blood of normal human beings and lower animals and in cultures of spleen and nodes of both human beings and lower animals. These observations suggest the universality of the giant cell in question and emphasize the ease with which it evolves in tissue culture.

In cultures of Hodgkin's disease nodes giant cells appear with such frequency and in such profusion as compared with those seen in cultures of non–Hodgkin's disease nodes that for Hodgkin's disease they may be indicative of some property or process peculiar to the disease. We doubt that the giant cell stimulus arises from the necrotic debris resulting from disintegration of lymphocytes and other cells, because there were many instances of widespread necrosis without giant cells having appeared. Nor can we agree that the giant cells arise as reactants against the coverslip on which the fragment is planted, for control material grown under identical conditions failed to produce reactions comparable to those seen in growing fragments of Hodgkin's disease nodes. For the same reason, too, we could not relate the occurrence of giant cells to the thickness of the clotted medium, nor to the size of the fragment. It would appear, therefore, that the substance responsible for evolution of the giant cells must be produced by the constituents of the tissue fragment. More work will have to be done to identify this substance. Evidence that it may be a virus is still weak; more plausible is the explanation that the giant cells arise in response to some metabolite originating in the tissue grown in vitro. Emphasis should be laid on "in vitro" metabolism, because the multinuclear giant cells in question were only rarely found in tissue sections of the original Hodgkin's disease nodes. Finally, the postulate that the giant-cell-stimulating substance is in any way related to the specific cause of Hodgkin's disease, namely, a virus, as suggested by Grand, is highly speculative and remains to be established and clarified.

SUMMARY

Multinucleated giant cells appear in cultures of explants from lymph nodes affected by many nonrelated diseases, likewise in cultures from fragments of spleen, and in cultures of blood cells from the buffy coats of centrifuged blood of both human beings and animals.

These cells are of the foreign body type and arise independently of the Sternberg-Reed cell. However, they occur in cultures of a greater number of nodes from Hodgkin's disease sources, in more fragments, in larger numbers, and are larger and have more nuclei, than do similar cells appearing in cultures of nodes from non–Hodgkin's disease sources. The giant cells in question, so conspicuous in tissue culture, are only rarely seen in histologic preparations of the source tissue.

These observations suggest that there is present in nodes affected by Hodgkin's disease a peculiar but still unknown factor which is made manifest in tissue culture by the advent of particularly large numbers of multinucleated giant cells of characteristically large size.

EXPERIMENTAL CORONARY SCLEROSIS

II. The Role of Infection in Coronary Sclerosis of Cockerels

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IN THE first paper in this series [1] a high incidence of "spontaneous" coronary sclerosis in White Leghorn and Barred Rock cockerels was reported. It was shown that the disease was initiated by a focus of degeneration and inflammation of the medial coat of the artery. Intimal proliferation occurred at the point of medial disease as a secondary reaction, and it was markedly accelerated when cholesterol was fed to the birds. The possible causes of the primary medial lesion were discussed, and it was suggested that bacteria or viruses, or allergenic products thereof, might be the agents.

The present report is concerned with the results of experiments which seem to eliminate infection of an acquired type as a cause of the primary lesion.

MATERIAL AND METHOD

The first experiment was designed to determine whether or not quarantine against communicable diseases would affect the incidence of coronary sclerosis in cockerels. Sixty 1 day old male White Leghorn chicks were obtained from the Dominion Experimental Farm, Ottawa, and were treated as follows:

Twenty chicks (group 1) were quarantined against infection. They were fed a standard starting ration until they were 3½ months old, and thereafter a standard growing ration to which 2 per cent cholesterol was added. All of the birds survived to the age of 7 months. They were then killed and their hearts examined for the incidence and the degree of coronary sclerosis.

Twenty chicks (group 2) were reared under ordinary laboratory conditions, without quarantine. They were fed the same type of starting ration and the same type of growing ration with added cholesterol as the birds in group 1.

This investigation was aided by a grant from the National Research Council of Canada.

From the Department of Medical Research, University of Western Ontario, London, Canada, and the Division of Animal Pathology, Dominion Department of Agriculture.

1. Paterson, J. C.; Slinger, S. J., and Gartley, K. G.: Arch. Path. **45**:306, 1948.

Nineteen of them survived to the age of 7 months. Then they were killed and their hearts examined for the incidence and the degree of coronary sclerosis.

Twenty chicks (group 3) were reared in quarantine as were those in group 1, and were fed rations identical to those given to birds in groups 1 and 2 except that cholesterol was not added. Thirteen birds survived to the age of 7 months. Then they were killed and their hearts examined for the incidence and the degree of coronary sclerosis.

Additional chicks (group 4), which were of various ages and breeds and each of which was less than 6 months of age at the start of the experiment, were obtained from the Dominion Experimental Farm, Ottawa, and were reared under ordinary laboratory conditions. These birds were fed the same diets as were those of group 3 (i. e., without added cholesterol). This group was included to obtain a general picture of the incidence of "spontaneous" coronary sclerosis of cockerels in the Ottawa district.

The rations used in this experiment were supplied by the Department of Poultry Husbandry of the Ontario Agricultural College, and they were identical in their constitution to those described in the first paper in this series.[1] However, before they were used, they were sterilized by autoclaving for fifteen minutes at 15 pounds' (6.5 Kg.) pressure. It was found that this form of sterilization destroyed some of the vitamins, and the deficiencies were made up by feeding unsterilized newly mown grass.

The method of quarantining and protecting groups 1 and 3 against introduction of an infectious agent was that usually followed in a virus unit and consisted in isolating the birds in special rooms and feeding them sterilized foods. In addition, the attendants wore rubber clothing, boots and gloves and had to pass through shower baths to reach the quarantine rooms, and there used a surgical mask.

The incidence and the degree of coronary sclerosis in individual birds were determined by procedures identical to those described in the first paper.[1] Serial sections were cut through the upper three fifths of each heart at intervals of 400 microns. With this technic, from 25 to 30 cross sections of the major coronary system, each showing at least three main arteries, were obtained from each specimen.

The second experiment was designed to determine whether staphylococcic toxin or living staphylococci of a strain virulent to chickens would initiate or accelerate medial lesions in the coronary arteries of chickens.

The staphylococcic toxin was obtained from the Connaught Laboratories, University of Toronto, and it was labeled "24 M.A., L.H. Potency = 0.045." It was injected intravenously into 4 white leghorn cockerels, each 6 months old, in doses varying from 0.5 to 2.0 cc. The injections were given five times at intervals of from three to four days. These 4 cockerels, together with three control birds of the same age, were killed from four to seven weeks after the first dose of toxin. Their hearts were sectioned serially, and the intoxicated and the control birds were compared with regard to the incidence and the degree of their arterial lesions.

The living staphylococci had been cultured one year previously from a joint cavity of a white leghorn cockerel which had died during an outbreak of staphylococcic septicemia in our experimental colony. This outbreak of staphylococcosis has been described in a previous communication,[1] and it was considered as a possible cause of the widespread coronary artery disease in the surviving birds. The staphylococci in question were passed through a rabbit in an attempt to enhance their virulence, and the viable material was then injected intravenously

into 12 white leghorn cockerels, each 2 months old. A single dose, varying from 1,000,000 to 100,000,000 organisms, was given to each cockerel. The birds died or were killed from five to twenty-one days after the injections, and their hearts, together with the hearts of 6 controls of the same age, were sectioned serially and examined for evidence of arterial disease.

OBSERVATIONS

The incidence of coronary sclerosis in the cockerels in our major experiment was found to be the same regardless of whether or not the birds had been quarantined or fed cholesterol. The data concerning each group are given in the table. It will be noted that approximately the same number of arterial lesions was found in quarantined and non-quarantined birds, both with and without cholesterol feeding. The

Effect of Quarantine on the Incidence and the Degree of Coronary Sclerosis in Cockerels

Procedure	Birds Examined	Sections of Heart Examined *	Incidence of Coronary Disease	Average Number of Affected Sections in Diseased Birds	Average Degree of Lesions in Affected Birds
Quarantine plus cholesterol....	20	519	14 of 20 (70%)	6	Moderate
Cholesterol only.....	19	487	13 of 19 (68%)	5	Moderate
Quarantine plus normal diet..	13	302	8 of 13 (62%)	6	Slight
Normal diet only †.....	20	435	12 of 20 (60%)	5	Slight

* Almost every section showed three or more cross sections of major coronary arteries.
† These birds were of various ages and older than the 7 months which was constant for members of the other three groups.

microscopic appearance of the lesions was identical with that described previously.[1] In cockerels on a standard diet the media was the site of hydropic degeneration and round cell infiltration, and the adjacent intima was thickened, vacuolated and rather fibrous. On the other hand, cholesterol-fed birds showed lesions of media and intima which were appreciably larger (but not more numerous), and these points were heavily infiltrated with foam cells.

A virulent strain of staphylococci or of staphylococcic toxin injected intravenously in repeated doses failed to initiate, or to accelerate, the lesions of coronary sclerosis in cockerels under the conditions of our second experiment. The incidence, the distribution and the degree of disease were the same in the treated birds as in the control birds. Individual lesions were encountered in the intoxicated group which were unusually severe (like that shown in the figure), but identical lesions were seen in control birds in the same experiment.

COMMENT

The present experiments seem to eliminate acquired infection as a cause of coronary sclerosis of chickens. The quarantine technic of our major experiment was admittedly imperfect; it cannot be compared, for example, with that used by Reyniers and associates [2] for rearing small animals under bacteria-free conditions. But, at the same time, it was probably rigid enough to reveal some difference in the incidence of

High power photomicrograph of a small coronary artery of a 6 month old White Leghorn cockerel which had been given intravenously five doses of staphylococcic toxin, of 2 cc. each, and killed one month after the first injection. The intima is thickened and vacuolated, and the underlying media shows marked necrosis of muscle fibers. A similar grade of necrotizing mesarteritis was noted in a control bird. Hematoxylin and eosin; × 300.

arterial disease in the various groups if acquired infection had been the source. This impression is supported by the results of our subsidiary experiments with staphylococcic infection, which gave entirely negative results.

Attention is therefore being directed elsewhere in attempting to elucidate the cause of coronary sclerosis of chickens. Congenital infection, particularly leukosis of fowl, is under consideraton, but even more emphasis is being placed on the possibility of hypersensitivity to dietary

2. Reyniers, J. A., and others: Rearing Germ-Free Albino Rats, Lobund Report, no. 1, University of Notre Dame, South Bend, Ind., 1946.

agents. The diets used in the experiments impress one with their extreme artificiality, and these same diets are in general use in the poultry industry today.

It is to be noted that cholesterol feeding did not affect the incidence or the distribution of arterial disease in birds in the present experiments, but it did accelerate the progression of individual lesions. This potentiating effect of cholesterol feeding on the course of "spontaneous" coronary sclerosis of chickens has already been described,[1] and is here confirmed in chickens bred in a different part of Canada.

SUMMARY AND CONCLUSIONS

White leghorn cockerels which had been quarantined against infectious disease until they were 7 months old failed to show any reduction of the incidence of coronary sclerosis as compared with that observed in control cockerels which had been reared under natural conditions. The intravenous injection of staphylococci of a strain virulent to chickens, and of staphylococcic toxin, failed to produce arterial lesions or to accelerate those of the spontaneous variety. The experimental findings suggest that infectious disease of an acquired type is probably not responsible for the medial lesions which initiate coronary sclerosis in white leghorn cockerels.

ROLE OF AGE IN ESTROGEN-INDUCED LYMPHOID TUMORS OF MICE

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THE AGE of the animals at the beginning of the treatment is one of the factors that determine the incidence of estrogen-induced mammary cancers in mice. According to the studies of Loeb,[1] there is in males a maximum susceptibility at about the time of onset of sexual maturity. In an extension of these investigations we [2] noted an age factor operative, though in a limited way, in estrogen-induced leukemia in castrated male mice of strain Dba. Subsequently, an age factor was shown to play a part in the incidence of leukemia caused by irradiation.[3] More recently, we [4] have studied the role of age in the production of mammary cancer in castrated and noncastrated male mice of strain C3H treated with an estrogen. Again, lymphoid tumors developed in a number of these animals, and it is on these findings and their dependence on age that we wish to report.

MATERIALS AND METHODS

Sixty-six male mice of strain C3H raised in our laboratory were castrated at the age of 3 to 4 weeks. Twenty-four of these castrates and 26 animals with intact testicles received subcutaneous injections of 0.03 mg. of alpha estradiol benzoate once a week.[5] This compound was dissolved in sesame oil and administered for five months beginning at the age of 4 to 5 weeks. Forty-two castrated and 33 noncastrated mice received the same treatment, but the injections were started when the animals had reached the age of 4 months. The mice were caged

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1. Loeb, L.: Biol. Symposia **11**:197, 1945.

2. Silberberg, M., and Silberberg, R.: Proc. Soc. Exper. Biol. & Med. **58**: 347, 1945.

3. Kaplan, H. S.: (*a*) Cancer Research **7**:141, 1947; (*b*) J. Nat. Cancer Inst. **9**:55, 1948.

4. Silberberg, M., and Silberberg, R.: Proc. Soc. Exper. Biol. & Med. **69**:438, 1948.

5. The Schering Corporation supplied progynon B.

in groups of 4 or 5 each. They were fed a stock diet of commercial chow and had water available at all times. The mice were examined at regular intervals, and sick-looking ones were killed. At necropsy, mammary glands, pieces of spleen, liver, lymph nodes, heart, lung, the endocrine organs and some bones were removed, fixed in 4 per cent formaldehyde and embedded in paraffin, and the sections were stained with hematoxylin and eosin. Mice that had died were inspected for gross changes, and if autolysis was not too far advanced, the internal organs were likewise taken out for histologic examination.

In this report the term "cancerous lymphoma" will be used to include lymphosarcoma as well as leukemia.

OBSERVATIONS

Cancerous lymphoma was not observed in 95 normal males of our C3H stock. Of 178 breeding females 8 to 21 months old, 2 showed

Summary of Experimental Data

Group	Experiment	Total No. of Mice	Animals Reaching Lymphoma Age			Animals in Which Cancerous Lymphoma Developed			
				Age, Mo.				Age, Mo.	
			No.	Mean	Range	No.	Percentage	Mean	Range
1	Noncastrated mice received estrogen from age of 1 mo.	26	19	12.8	9-17	4	21.1	15.0	14-17
2	Castrated mice received estrogen from age of 1 mo.	24	19	13.2	9-18	6	31.6	13.5	9-17
3	Noncastrated mice received estrogen from age of 4 mo.	23	14	13.5	9-20	1	7.1	18.0	.....
4	Castrated mice received estrogen from age of 4 mo.	42	34	13.9	9-19	2	5.9	18.0	17-19

cancerous lymphoma at the age of 19 months. A few old breeders had moderately enlarged mesenteric lymph nodes.

The results of our experiments are presented in the table. The numbers of mice in which cancerous lymphoma developed in the various experimental groups and the ages at which death occurred are recorded. A correlation between the occurrences of mammary cancer and cancerous lymphoma could not be established. Animals referred to as "reaching the lymphoma age" are those that lived nine months and more, lymphoma not having been observed before the age of 9 months.

Group 1 (mice with intact testicles receiving estradiol benzoate from the age of 1 month on).—Of a total of 26 animals, 19 reached the lymphoma age. The mean age at death of all animals in this group was 12.8 months, with a range from 9 to 17 months. In 4 of them (21.1 per cent) cancerous lymphoma was found at a mean age of 15 months, the age range being 14 to 17 months.

Group 2 (mice castrated at the age of 3 to 4 weeks and treated with estradiol benzoate from the age of 1 month on).—Of 24 animals, 19 lived

to reach the lymphoma age. The mean age at death was 13.2 months, with a range from 9 to 18 months. In 6 of these mice (31.6 per cent) cancerous lymphoma developed, and they either died or were killed at a mean age of 13.5 months. The youngest animal with cancerous lymphoma in this series was 9 months old, the oldest 17 months old.

Group 3 (mice with intact testicles receiving estradiol benzoate from the age of 4 months on).—Of 33 mice, 14 reached the lymphoma age, the mean age at death being 13.5 months, with a range from 9 to 20 months.[6] One animal (7.1 per cent) died with cancerous lymphoma at the age of 18 months.

Group 4 (mice castrated at the age of 3 to 4 weeks and treated with estradiol benzoate from the age of 4 months on).—Of 42 mice, 34 reached the lymphoma age. The mean age of these animals at death was 13.9 months, with a range from 9 to 19 months. In 2 of these mice (5.9 per cent) cancerous lymphoma appeared at the age of 17 and 19 months, respectively.

In the experimental mice the diagnosis of cancerous lymphoma could be made after examination of the gross specimen in most cases. In some animals soft, whitish mediastinal tumors originating in the thymus were seen filling a large part of the thoracic cavity; in others similar neoplasms were found in the mesentery or attached to the intestine. The cervical, mediastinal, mammary and para-aortic lymph nodes and the liver were invariably enlarged; the spleen was from four to six times the usual size, and firm and granular on the cut surface. Liver and kidney showed an ochre brown color, and the lungs were pale. In estrogen-treated animals with or without cancerous lymphoma an oblong, moderately firm, reddish mass, varying in size from about 7 by 3 by 3 mm. to 15 by 5 by 5 mm., was frequently noted in the mesentery near the pancreas.

HISTOLOGIC OBSERVATIONS

The macroscopic diagnosis could readily be confirmed in microscopic sections. The usual structure of the lymph nodes, and to a varying degree that of the spleen, was obliterated by diffusely invading cancerous cells of the lymphocytic series. Liver, kidney, lungs and heart likewise showed numerous areas of this cellular infiltration (figs. 1 to 3). However, the involvement of the various organs and the size of the individual foci differed considerably. In the liver the earliest changes consisted of

6. The high mortality of these animals was due to the frequent occurrence of calculosis of the urinary bladder and advanced pyelonephritis. Many of these mice died suddenly, without previous evidence of illness, and the organs were too autolyzed to permit adequate histologic examination. Owing to the small number of animals available for study, the results do not lend themselves readily to statistical evaluation. However, in view of the fact that the findings were similar to those in the following group they are probably significant.

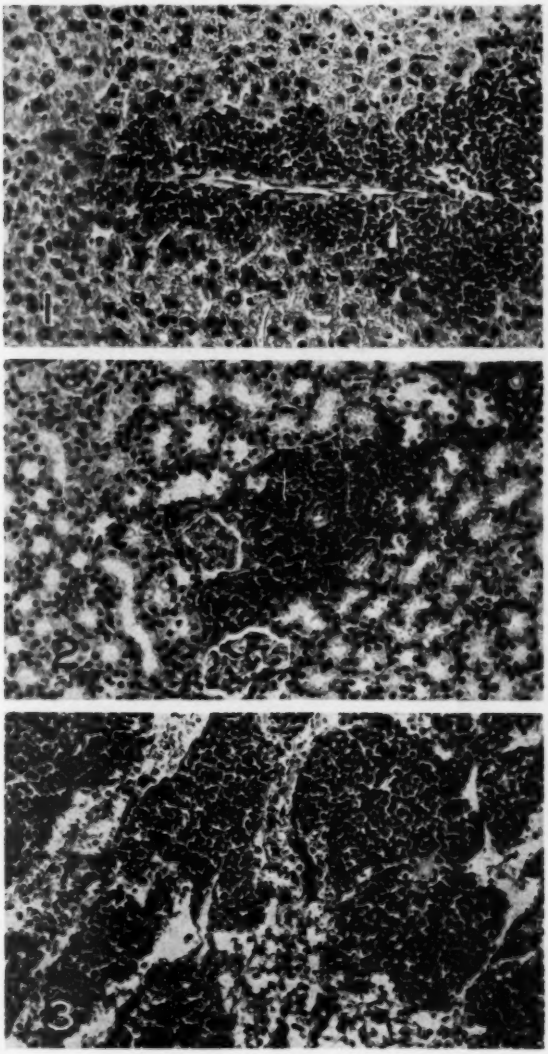

Fig. 1.—Leukemic infiltration of the liver of a 14 month old castrated male mouse which had been given injections of estradiol benzoate for five months from the age of 1 month on; × 180.

Fig. 2.—Cancerous lymphoma of the kidney of a 9½ month old castrated mouse which had been given injections of estradiol benzoate for five months from the age of 1 month on; × 180.

Fig. 3.—Cancerous lymphoma of a mesenteric lymph node of the mouse whose renal involvement is shown in figure 2; × 180.

widely scattered small perivascular foci composed of a few immature blood cells, some of which showed mitotic figures. Megakaryocytes were seen here and there. In more advanced cases, the cellular infiltration was more extensive, and more organs were affected. In leukemic animals, immature white blood cells were seen in the sinusoids of the liver and in the blood vessels. The cancer cells were usually of the

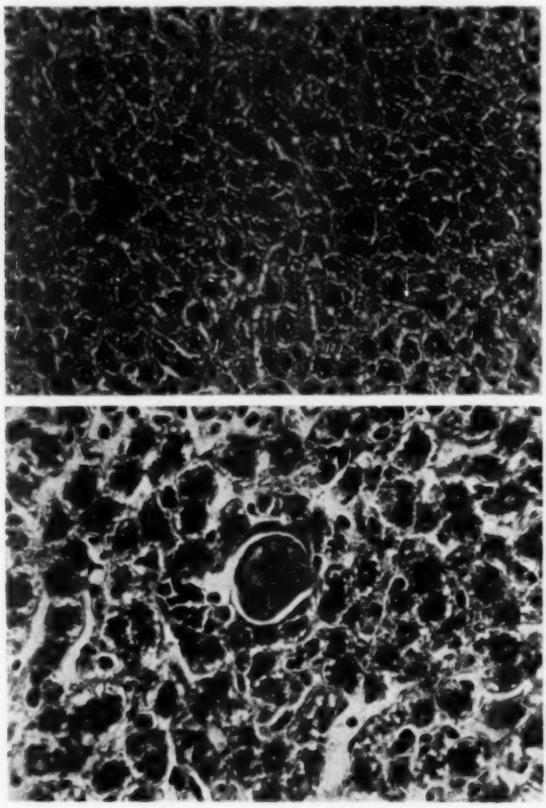

Fig. 4.—Extramedullary hemopoiesis in the liver of a 16 month old male mouse which had been given injections of estradiol benzoate from the age of 1 month on, for five months; × 180.

Fig. 5.—Focus of extramedullary hemopoiesis in the liver of a 14 month old male mouse which had been given injections of estradiol benzoate from the age of 1 month on, for five months; × 360.

erythrocytic series (rubriblasts) or the lymphocytic series. Only once was myelogenous leukemia found with a variety of myeloid cells infiltrating the tissues.

In about one third of the animals, hemopoiesis was found in spleen, lymph nodes, kidney and liver (figs. 4 and 5). The hemopoietic foci were small and scattered through these organs; they consisted predominantly of cells of the granulocytic and erythrocytic series and megakaryocytes.

The mesenteric mass was unrelated to cancerous lymphoma, since it occurred not only in animals in which lymphoma had developed but in those in which this tumor had not been observed. The mass was composed of normal or cancerous lymphatic tissue with dilated, engorged sinuses of varying size and distribution (figs. 6 and 7). These structures are apparently identical with those observed by Gardner and co-workers [7] in mice of the same strain. In some animals there was a diffuse but moderate dilatation of the sinuses; in others the engorgement was more localized, and the individual spaces had reached considerable size. Laminated thrombi were frequently present in the lumens (fig. 8). The lining endothelium was regular throughout and showed no evidence of proliferation. Thus, in agreement with the view of Gardner,[7] these formations are not to be considered as neoplastic. Whether they represent an analogue to the hypervolemia of the liver occurring after transplantation of granulosa cell tumors [8] remains to be decided.

COMMENT

In the following paragraphs the present findings will be discussed and compared with earlier results obtained in similar experiments in male mice of strain Dba.

In males of strain C3H receiving injections of estradiol benzoate from the age of 1 month on, castration raised the incidence of cancerous lymphoma from 21.1 to 31.6 per cent. In corresponding males of strain Dba the incidence of lymphoma was 30 per cent in the noncastrates and 50 per cent in the castrates. In strain C3H the mean age at death of mice with cancerous lymphoma was 15 months in the noncastrates and 13.5 months in the castrates; in strain Dba the mean age at death of lymphoma was 11.6 months in the noncastrates and 11.3 months in the castrates.

In mice of strain C3H orchidectomized at 3 to 4 weeks of age and given injections of estradiol benzoate from the age of 4 months on, the

7. Gardner, W. U.; Dougherty, T. F., and Williams, W. L.: Cancer Research **4**:73, 1944.

8. Furth, J., and Sobel, H.: (*a*) J. Nat. Cancer Inst. **7**:103, 1946; (*b*) Science **105**:41, 1947. Gardner.[7]

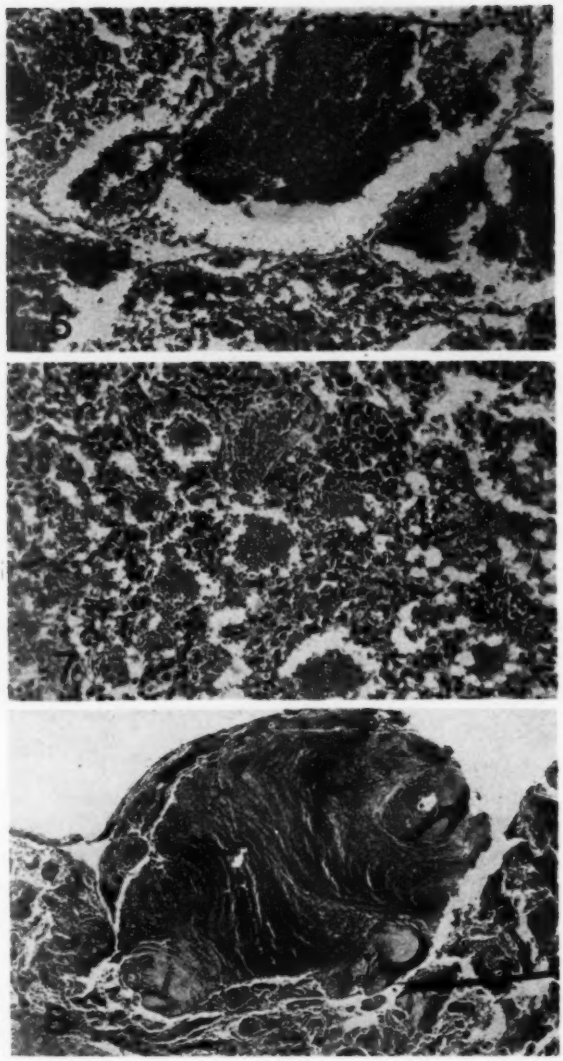

Fig. 6.—Enlarged sinuses of a mesenteric lymph node of a 16 month old male mouse which had been given injections of estradiol benzoate from the age of 1 month on, for five months; × 95.

Fig. 7.—Diffuse enlargement of sinuses of a mesenteric lymph node of a 13 month old castrated mouse which had been given injections of estradiol benzoate for five months from the age of 4 months on; × 180.

Fig. 8.—Large thrombus in a dilated sinus of mesenteric lymph node of a 17 month old castrated mouse which had been given injections of estradiol benzoate for five months from the age of 1 month on; × 95.

incidence of lymphoma was 5.9 per cent; it was 7.1 per cent in the noncastrated mice of this age group. The mean age at death was 18 months for both the castrated and the noncastrated animals. The estrogen administered to mice of strain Dba had in the older age group produced cancerous lymphoma in 43.5 per cent of both the castrated and the noncastrated mice, the mean age at death being 11.6 months in the former and 12.9 months in the latter.

A discrepancy seems to exist as regards the incidence of cancerous lymphoma in the older groups of noncastrates of strain C3H and Dba. In noncastrates of strain C3H treated from the age of 1 month on, the incidence of lymphoma was 21.1 per cent; in those treated from the age of 4 months on, it was 7.1. In corresponding groups of Dba males this decline was not observed. On the contrary, the younger series showed a 30 per cent and the older one a 43.5 per cent incidence of lymphoma. There was, however, a difference of about ten days in the age at which castration was performed and estrogen treatment was begun in the two strains: The mice of strain Dba were about 3 weeks and those of strain C3H about 4½ weeks old at the beginning of the treatment. This change in the procedure was necessary because of the high mortality of our young C3H males. While an age difference of ten days seems small, it may suffice to influence the results of the experiments as shown by Loeb and co-workers [9] in experiments on the role of age in estrogen-induced breast cancer: The tumor incidence of mice receiving estrogen from the age of 2 weeks was lower than that of mice treated from the age of 4 weeks on. Similar conditions have recently been observed in regard to lymphoma induced by irradiation.[3] As in the aforementioned experiments,[9] a maximum susceptibility to estrogen induction of lymphoma may occur at or about sexual maturity. Our C3H mice were apparently close to this stage, whereas our Dba mice might not yet have reached the peak of susceptibility. In strain Dba a slight increase in the susceptibility would have sufficed to raise the incidence of cancerous lymphoma from the 30 per cent in the younger group to or above the 43.5 per cent found in the older series.

These findings allow several conclusions: (1) In young males of both strains the testicle exerts an inhibiting effect on the production of cancerous lymphoma by estrogen if treatment is begun at 3 to 5 weeks of age; (2) in neither strain does the absence of the testicle change the incidence or the time of appearance of cancerous lymphoma if the estrogen treatment is started three months after castration. The role played by the testicle in estrogen induction of cancerous lymphoma in one of the two strains used is thus similar to its role in the other.

9. Loeb, L.; Suntzeff, V.; Burns, E. L., and Schenken, I. R.: Arch. Path. **38**:52, 1944. Loeb.[1]

However, differences were observed with regard to the influence of the age factor in experimental leukemogenesis. In castrates of strain C3H treated at an early age cancerous lymphoma was about five times as frequent (31.6 per cent) as in the castrates treated from the age of 4 months on (5.9 per cent). Therefore in strain C3H an extratesticular factor aids in determining the incidence of cancerous lymphoma produced by estrogen. This factor may reside within the hemopoietic tissues themselves and may consist of decreasing responsiveness to leukemogenic stimuli with advancing age. The aging hemopoietic tissue of males of this strain would thus act as does the mammary gland in response to administration of estrogen.

In contrast to conditions in strain C3H the influence of age, if any, on estrogen induction of cancerous lymphoma in strain Dba is of minor significance. Fifty per cent of the castrates given injections from the age of ½ month on and 43.5 per cent of those treated from the age of 4 months on showed cancerous lymphoma. This strain difference in regard to the role of the age factor may possibly be connected with the difference susceptibility of the two strains to estrogen induction of lymphoma.[10] In strain C3H, the incidence of estrogen-induced cancerous lymphoma was lower in all groups, and the mean age at death (13.5 to 18 months) was higher than in strain Dba (11.1 to 12.9 months). This indicates a lesser susceptibility of strain C3H as compared with that of our strain Dba. The more marked the susceptibility to exogenous stimulation—or the more powerful the stimulus—the less significant may become the role in leukemogenesis of secondary factors, such as age, and perhaps even that of the testicles. In the highly susceptible strain Dba neither the removal of the testicles nor the age of the animals noticeably affected the incidence and time of appearance of cancerous lymphoma as they did in strain C3H. In the latter strain, by contrast, the lower susceptibility might have permitted the relatively weak secondary factors to assert themselves and to exert a more obvious influence on the incidence of cancerous lymphoma. Whether this suggestion might apply also to estrogen-induced mammary cancers remains to be decided. Our findings on the significance of age in the development of estrogen-induced breast cancers of C3H males would support this point of view[4]: In castrated males of strain C3H receiving estradiol benzoate from the age of 1 month on, the incidence of mammary cancers was 44.4 per cent; it was 30 per cent in castrates given injections from the age of 4 months on. Thus the incidence of mammary cancer dropped only slightly (about 33 per cent) as the age at the beginning of the estrogen treatment advanced. These same groups of males, however, showed an incidence of lymphoma of

10. Kirschbaum, A.: Yale J. Biol. & Med. **17**:163, 1944.

31.6 and 5.9 per cent respectively. The age difference in the two groups thus caused an impressive (about 400 per cent) decrease in the incidence of cancerous lymphoma in the older group. Therefore, the age factor exerted a more obvious influence on estrogen induction of leukemia than on estrogen induction of mammary cancer. Since strain C3H is more susceptible to the development of cancer of the breast than to that of cancerous lymphoma, the role of the age factor seems to be inversely proportional to the degree of susceptibility.

SUMMARY

The susceptibility of male mice of strain C3H to estrogen induction of cancerous lymphoma varied with the age at which the administration of the estrogen (alpha estradiol benzoate) was begun. A maximum susceptibility was found about the onset of sexual maturity. Castration performed at the age of 3 to 4 weeks raised the incidence of cancerous lymphoma if the treatment with estradiol benzoate was started one week after orchidectomy. Castration failed to influence the incidence of cancerous lymphoma if the estrogen treatment was begun at the age of 4 months. In castrates receiving the estrogen from the age of 4 months on, the incidence of cancerous lymphoma was significantly lower than in castrates treated with estradiol benzoate from the age of 1 month on. Thus an extratesticular age factor, presumably residing in the aging hemopoietic tissues, aids in determining the outcome of this type of leukemogenesis. The significance of the age factor varies in different strains and seems to decrease as the genetic susceptibility of the animals to estrogen induction of cancerous lymphoma increases.

DEVELOPMENT OF ANAL DUCTS AND GLANDS

With Reference to the Pathogenesis of Anorectal Disease

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IT HAS been assumed by certain investigators that the anal crypts and contiguous ducts and glands provide a route by which infection is transmitted to the deeper tissues of the anal region. The anal crypts, ducts and glands have thus been considered as a factor in the genesis of anal fissures, abscesses and fistulas. The possibility that the ducts of the anal glands may become obstructed or that the distal portion of uncanalized glands may have secretory activity could easily explain the development of cysts and of those abscesses and fistulas which do not communicate with the anal canal.

In a previous study of the anal ducts and glands Hill, Shryock and ReBell [1] observed that these structures varied widely in number and in the depth to which they penetrated into the submucosal and muscular tissues. These observations were based on 17 specimens from: a 7 month fetus, 5 newborn infants and 11 adults. It was observed that in some specimens from newborn infants the ducts lacked complete canalization and that in 1 of the adult specimens there was a simple retention cyst of the tubular part of an anal gland. It was reported that smooth muscle occurred uniformly in the submucosa in adult specimens, but that in specimens from newborn infants the smooth muscle was merely in the process of differentiation.

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This investigation was aided by a grant from the Alumni Research Foundation of the College of Medical Evangelists and the technical assistance of Harold Messinger.

1. Hill, R.; Shryock, E. H., and ReBell, F. G.: J. A. M. A. **121**:742, 1943.

Although that study supported the concept that the anal glands play a definite role in the genesis of anorectal disease, it also raised several questions, the answers to which were dependent on a detailed study of the embryonic development of the anal glands. Previous studies of the development of these glands have been based on a small number of specimens and have therefore not permitted accurate conclusions with respect to the number of anal glands present in the various fetal age groups.

The present study was undertaken, therefore, in the hope of finding answers to the following questions: Do anal glands tend to grow deeper throughout the stages of fetal development? What percentage of anal glands penetrate the muscularis? Do the anal glands canalize progressively? Does an obstructed gland or duct represent one which has not yet canalized or one which has become secondarily obstructed? What is the time relation between the differentiation of the muscularis mucosae and the development of the anal glands? When does the "muscularis submucosae ani" develop?

REVIEW OF THE LITERATURE

Johnson [2] (1914) was the first to publish accurate information with respect to the detailed structure and location of the anal glands. His study was based on serial sections and reconstructed scale models. In his review of the literature, Johnson acknowledged the earlier contributions of Herrman and Desfosses [3] (1880) and of Herrmann [4] (1880), who were presumably the first workers to differentiate the anal glands (intramuscular glands) from the anal crypts and from the circumanal glands. Herrmann and Desfosses observed that some of the anal glands penetrated the internal sphincter. Herrmann [4] (1880) was unable to find any trace of secretory epithelium in these "special acinous glands" or intramuscular glands and therefore admitted that they might be simply sinuses rather than true glands.

Braun [5] (1901) was unable to find the anal glands described by Herrmann and therefore completely denied their presence. Johnson [2] stated that these tubular structures resembled glands and were lined with 2 to 3 layer cuboidal epithelium, but he found no evidence that the epithelium was glandular in character. However, Hill, Shryock and ReBell [1] (1943) presented evidence in their study that at least some portions of these structures were definitely glandular. They were able to demonstrate cells showing apical cytoplasmic vacuoles and found that these cells stained for mucin with Krajian's carbolfuchsin method.

2. Johnson, F. P.: Am. J. Anat. **16**:1, 1914.

3. Herrmann, G., and Desfosses, L.: Compt. rend. Acad. d. sc. **90**:1301, 1880; cited by Johnson.[2]

4. Herrmann, G.: J. de l'anat. et de physiol. **16**:434, 1880; cited by Johnson.[2]

5. Braun, W. O.: Untersuchungen über das Tegument der Analöffnung, Inaug. Dissert., Königsberg, R. Leupold, 1901, pp. 1-50; cited by Johnson.[2]

Tucker and Hellwig [6] (1935) also referred to these structures as glandular ducts and stated that they had observed them developing from the upper portion of the zona intermedia in the 30 mm. embryo.

Bremer [7] (1936) stated that the secretory cells of the anal ducts disappeared in postnatal life, but Hill and associates [1] presented photographs of a cyst of an anal gland from an adult which implied that secretory activity had persisted.

Questions have been raised as to the direction of penetration of the glands. Johnson [2] stated that they may extend either cephalad or caudad. This was confirmed by Hill and his group, who found that most of the glands penetrated laterally and caudad but that a few extended in a cephalad direction. The study of Kratzer and Dockerty [8] (1947), however, did not add confirmatory evidence. Their study of serial sections of an 8 month stillborn boy indicated that there was no penetration upward. Most writers agree that lateral penetration may extend to or through the internal sphincter.

A variation in the degree of canalization of the intramuscular glands was noted by Johnson [2] and by Hill, Shryock and ReBell.[1]

Harris [9] (1929) emphasized that certain of the intramuscular glands penetrated deeply and reached the inner circular layer before the muscularis mucosae had differentiated. This is apparently substantiated by the findings of Johnson [2] and Tucker and Hellwig.[6] The latter referred to the glandular ducts which were present in the 30 mm. embryo. Johnson stated that in the 190 mm. embryo the muscularis mucosae was absent from the anal region but that at birth it was completely formed and was prolonged in several strands which extended down into the rectal columns.

Fine and Lawes [10] (1940) called attention to the constant presence of muscularis submucosae ani in the region of the pecten band. The deepest of these smooth muscle fibers were in close contact with the internal sphincter, while the superficial ones were said to be continuous with the muscularis mucosae of the rectum. The fibers were sometimes scattered and sometimes gathered into definite muscle bundles. All the specimens investigated were from adults.

The present study of the anal canal of the fetus provides answers for some of the questions, hitherto unanswered, concerning the development of these various structures.

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6. Tucker, C. C., and Hellwig, C. A.: Arch. Surg. **31**:521, 1935.
7. Bremer, J. L.: A Textbook of Histology Arranged upon an Embryological Basis, ed. 5, Philadelphia, P. Blakiston's Son & Co., 1936, p. 296.
8. Kratzer, G. L., and Dockerty, M. B.: Surg., Gynec. & Obst. **84**:333, 1947.
9. Harris, H. A.: Proc. Roy. Soc. Med. **22**:1331, 1929.
10. Fine, J., and Lawes, C. H. W.: Brit. J. Surg. **27**:723, 1940.

MATERIALS AND METHODS

A microscopic study was made of the anal canals of 49 fetuses. These fetuses ranged in crown-heel length from 9.5 to 38 cm. Serial sections were made of each anal canal. All sections were stained in a routine manner with hematoxylin and eosin.

Scale models were made of 4 selected specimens from 12 cm., 17.5 cm., 23 cm. and 30.5 cm. fetuses. The models were constructed by the laminated cardboard technic; serial sections were projected and traced on cardboard, and the tracings were then cut out and assembled in a serial sequence.

OBSERVATIONS

Anal glands were found in 26 (53 per cent) of the 49 fetuses studied. No glands were found in specimens from fetuses less than 16 cm. in length (about four months' gestation).

The glands varied with respect to depth of penetration (fig. 1). Some extended only to the submucosa; others, to the inner circular layer of muscle, and the deepest ones were found in the external longitudinal layer of muscle. Seventy-seven per cent of all the glands seen penetrated into the muscularis.

The number of glandular elements per specimen varied from 1 to as many as 13. The average number of glands and/or ducts in the submucosa only was 3.7 per specimen. The average number in the muscularis was 5.4.

The reconstruction of the specimen from the 30.5 cm. fetus clearly showed the complex branching of the terminal portions of some of the glands (fig. 2). It was also evident from the model that some of the terminal branches extended in a cephalad direction.

The question of canalization of the glands was investigated. Four specimens presented one uncanalized gland and 1 showed two uncanalized glands. Canalization does not necessarily progress peripherally. In 1 case (fig. 3) a gland was demonstrated to be uncanalized proximally and distally but well canalized in its more central part. Surrounding the uncanalized segments there was no evidence of inflammation.

All glands were found to be connected with the anal canal.

The muscularis mucosae had not differentiated in fetuses under 16 cm. in length. It was quite constantly present in fetuses above 24 cm. but was not well differentiated in all specimens.

GENERAL CONSIDERATION

The observations incident to the present study suggest that the anal glands are not easily distinguished until about the fourth month of intrauterine life. Even in cases in which differentiation may have been initiated at an earlier age, there is no penetration of the muscularis until about the fourth month.

In view of the earlier report [6] of Tucker and Hellwig it was surprising to find that no glands occurred in the 10 specimens from fetuses under

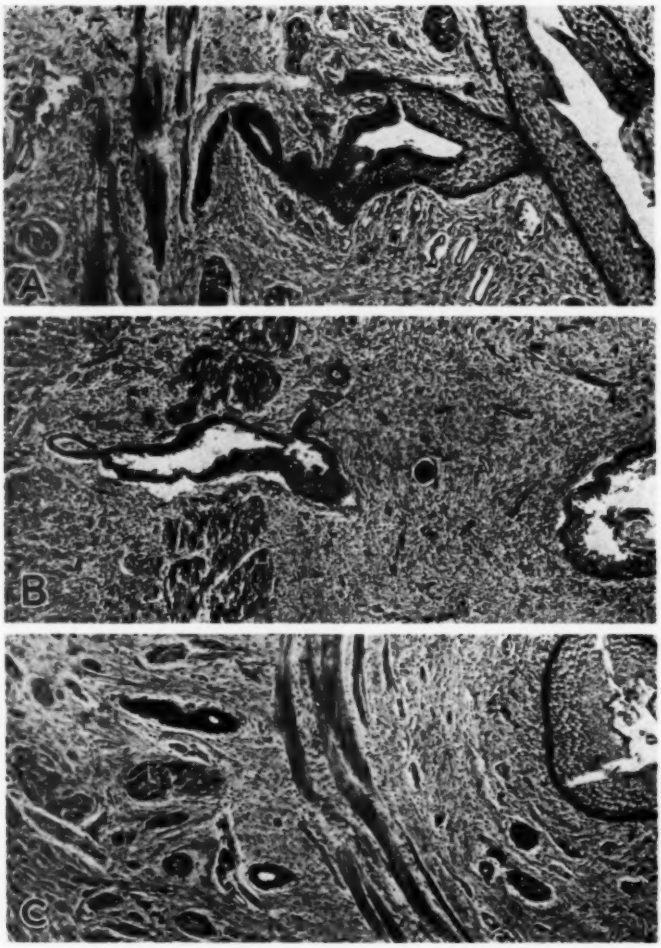

Fig. 1.—Anal glands penetrating to varying depths: *A*, into the submucosa (× 100); *B*, through the circular muscle layer (× 70); *C*, into the longitudinal muscle layer (× 70). Hematoxylin and eosin stain.

16 cm. in length. This finding was borne out not only by microscopic study but also by the reconstructed model of the specimen from the 12 cm. fetus.

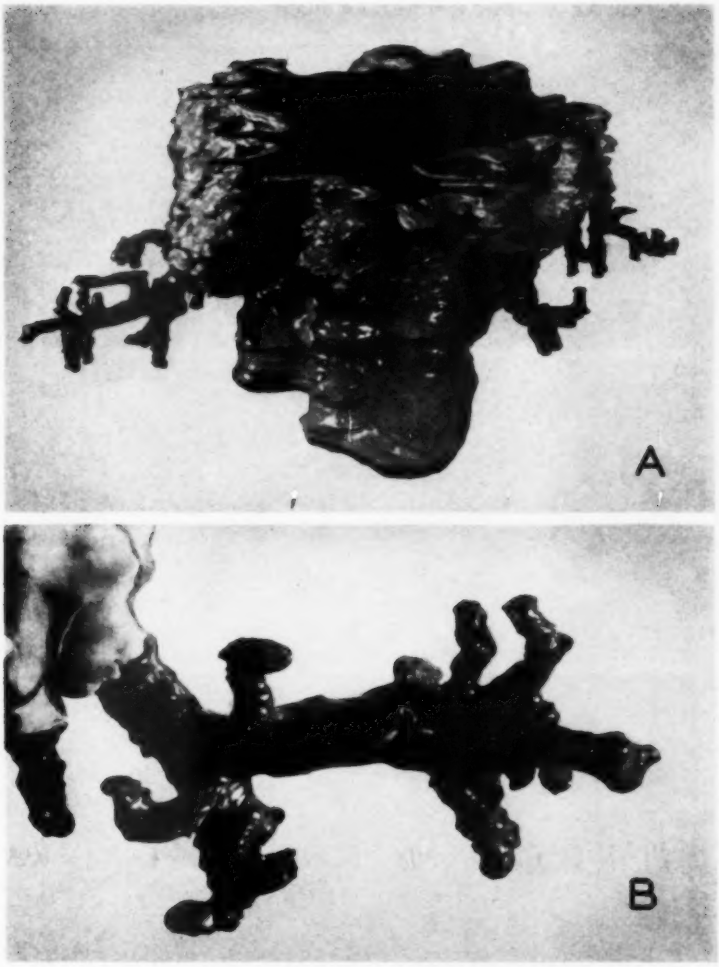

Fig. 2.—*A*, reconstruction of the anorectal region from a 30.5 cm. fetus showing numerous glands and their complex branching; *B*, single glandular element (enlarged) of the anorectal region from a 30.5 cm. fetus showing branching cephalad as well as caudad.

The anal canal of the fetus presents a number of longitudinal folds, which may be so complex in contour as to be confused with rudimentary anal ducts or glands. Positive identification requires the sequential

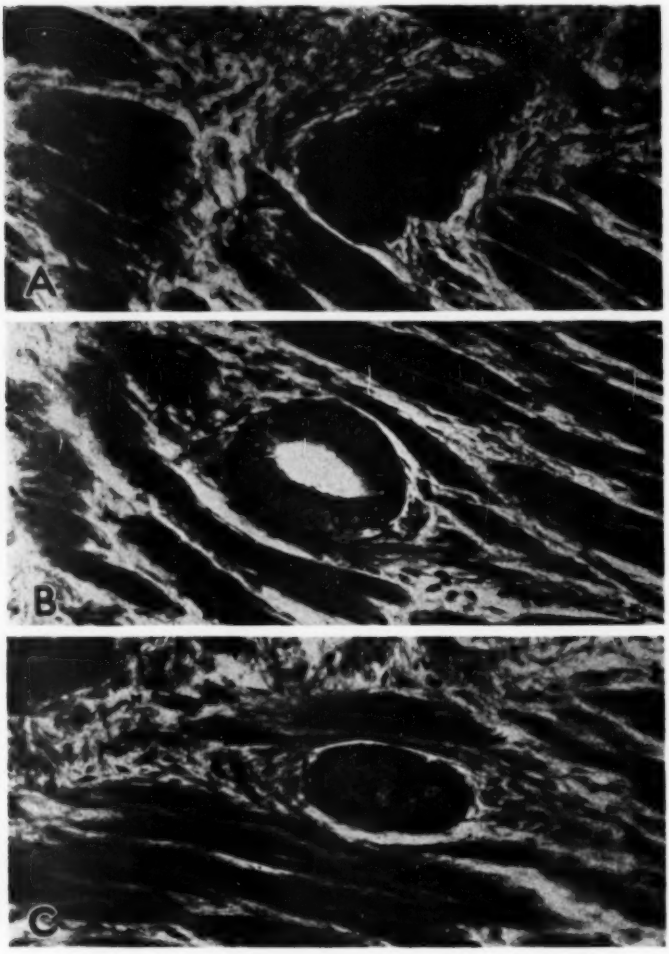

Fig. 3.—Sections through an anal gland showing incomplete canalization: *A*, proximal closed portion (× 270); *B*, middle open portion (× 270); *C*, distal closed portion (× 270). Hematoxylin and eosin stain.

study of serial sections and preferably recourse to a scale model. In several of the specimens the mucosa was folded in such a way as to

resemble, in a single microscopic preparation, the developing ducts which Tucker and Hellwig observed in their specimen from a 30 mm. fetus. Detailed study of the serial sections indicated, however, that those features were only complex folds and not ducts or glands. It must be admitted that in specimens in which the glands penetrate but a short distance, as into the submucosa only, it is difficult to be sure whether a given structure is a duct or a simple fold, for the epithelium lining a confirmed duct may appear much like that lining the zona intermedia of the anal canal. Differentiation becomes simple, however, when a gland is seen penetrating the muscularis.

It was interesting to note that so many (77 per cent) of all the glands seen penetrated into the muscularis. This study did not suggest any correlation between fetal age and depth of gland penetration. It has been suggested that the ducts and glands form avenues for the spread of infection. This deep penetration seems to give a reasonable explanation of certain rectal and perirectal lesions.

Certain of the terminal branches of the glands were found to penetrate cephalad as well as caudad (fig. 2), thus confirming the earlier work of Johnson [2] and of Hill, Shryock and ReBell.[1] If these cephalad branches become infected, sinus tracts result which may extend variable distances up the wall of the rectum. These may again communicate with the lumen and thus establish complete fistulas.

Embryologically, all the glands are epithelial outgrowths of the anal mucosa, but because of incomplete canalization a limited number may never actually communicate with the anal canal. Thus it is possible that a gland such as shown in figure 3 might develop into a cyst if the proximal portion remained uncanalized.

The observation that some specimens do not have glands and that those which do may show considerable variation in the number of glands present is important from the standpoint of the spread of infection. Those persons with a large number of glands are more susceptible than are those with few or no glands. It would seem, therefore, that a certain percentage of persons are predisposed to perirectal and perianal complications by reason of the development of intramuscular glands in the anorectal region.

A search was made for muscle fibers in the submucosa which might conform to the description given by Fine and Lawes [10] of what they termed muscularis submucosae ani. Hill and associates [1] stated that in the newborn infant the smooth muscle of the submucosa of the anal canal is in a process of differentiation, muscle cytoplasm being obvious only in scattered areas. In the present series we did not find anything that we thought could be called muscularis submucosae ani. None of the specimens were from fetuses larger than 38 cm.; so one would infer that the muscularis submucosae ani must differentiate later in fetal life.

In our study it became evident that the muscularis mucosae does not differentiate at any definite age. It was present in a specimen from a 16 cm. fetus and not in a specimen from a 35 cm. fetus. However, it apparently never differentiates before the third or fourth month of intrauterine life. In view of this one cannot state positively that the glands develop before the muscularis mucosae or vice versa. However, the poor differentiation of the muscularis mucosae in the early fetal specimens suggests that it usually develops later.

SUMMARY AND CONCLUSIONS

A microscopic study has been made of the anal canals of 49 fetuses ranging in crown-heel length from 9.5 to 38 cm. Laminated models were made of selected specimens. Photographs of models and microscopic sections are presented herewith.

Particular attention was given to the time of differentiation of the anal glands, the muscularis submucosae ani and the muscularis mucosae. The depth and the direction of penetration, as well as the canalization, of the glands were investigated.

Anal glands were found in 53 per cent of the specimens studied. None were found in specimens from fetuses under 16 cm. long. In the majority of instances the glands had penetrated the muscularis, thus providing an avenue by which infection might spread to deeper layers of the anorectal tissues.

The number of anal glands present in a single specimen varied from 1 to 13. It is believed that those persons who have a relatively large number of glandular elements have a greater predisposition to perianal and perirectal pathologic conditions, which probably are due to infection entering and spreading through these channels.

Six incompletely canalized glands were found. If the proximal portions remain uncanalized, the distal parts may develop into cysts, which predispose to abscess and fistula formations having no communication with the anorectal canal.

The muscularis submucosae ani was not identified as a definite structure in any specimen of this series. Presumably, it develops after the seventh month of intrauterine life.

The muscularis mucosae does not develop prior to the third or the fourth month of intrauterine life, and even as late as the seventh month it may be poorly differentiated.

DISCUSSION

DR. C. C. TUCKER, Wichita, Kan.: It has become established that anal ducts play an important role in infection arising in and around the anal-rectal line. In the past twelve years the number of specimens which my associates and I have examined microscopically have run into the thousands. Only in rare instances

have we found that these ducts were not infected. The infections observed ranged from the chronic to the acute stage. We have also noted that the pathologic process is confined to ducts which open into the crypts of Morgagni; that the ducts are natural incubators for the colon bacillus, the staphylococcus, the gonococcus, the streptococcus and, in rare instances, the tubercle bacillus. That these ducts are preformed structures and not results of an ulcerative process was demonstrated by us on dissecting specimens without inflammatory changes. We found these ducts sometimes extending into the internal sphincter muscle. They are either simple tubular ducts or the more complex branching structure which extends from the mucosa of the anal canal into the submucosa or the muscular layer.

The invading bacteria pass through the crypts of Morgagni into the ducts and attack the epithelial cells lining the lower parts of the ducts; the epithelium changes to columnar cells. The crypts themselves are lined by stratified squamous cells which are much more resistant to bacterial infection except in gonococcic infection of the female. Because of the anatomic structure of the anus, the parts become bathed in the gonorrheal discharge at the time of defecation; thus the crypts of Morgagni become infected as well as the ducts. In fistulas, we believe, the origin of infection is confined to the anal ducts.

Drs. Hill, Small, Hunt and Richards are to be commended on the work they have done on the embryonic development of anal ducts and glands.

Time and space will not permit me to go into the embryologic phase of this paper, but I hope that in the future I shall have the privilege of doing so.

Dr. J. P. Nesselrod, Evanston, Ill.: My associates and I became interested in this problem of the role played in anal infection by the anal glands and ducts. Our work differs in this way: The anatomic material is adult and not fetal. Therefore we are more likely to find, as we did in our work at Northwestern University, considerable round cell infiltration of the tissues that are involved. Contrary to the observations of Hill and his associates I was unable to demonstrate, except in an occasional instance, glandular invasion of the muscular structures. Whether this is due to the difference in our anatomic material I do not know. We may not agree exactly in our ideas of these glands, but I believe we are driving at the same thing and that is anal infection and its role in the pathogenesis of anorectal inflammatory disease. By anal infection is meant the sum total of events taking place in the development or the pathogenesis of anorectal inflammatory disease, and I include not only those entities mentioned by Dr. Hill and his associates but also hemorrhoids.

This work of Hill and his associates affords another striking demonstration of the contribution made by the basic sciences to the everyday work of the clinician and the surgeon. This concept of anal infection and its role in anorectal inflammatory disease marks a prominent step forward in understanding of these problems. Only at such time as the practicing clinician becomes aware of this process will he be able to give his patient the proper advice. We will then relegate the so-called shortcut methods to their proper place as compared with adequate surgical management.

Dr. Edward Levy, New York: All can congratulate the authors for a masterly presentation. In my own anatomic studies I came across a paper submitted by a German for the doctor's degree about 1867, in which he described an unusual or abnormal form of crypts, and in the illustrations accompanying the paper he showed crypts about the size of those which I have seen in the microscopic slides of Dr. Hill's demonstration at the exhibition. Following that, one waited until about 1914, when Johnson did his work on the embryonic anorectal canal, and for the first time a reconstruction of intramuscular crypts was demonstrated. There

is available now, in Baltimore, Johnson's reconstruction of glands, which he observed in the embryo, penetrating for a variable distance from the crypts into the deep tissue.

About 1937 Dr. Tucker presented before the American Proctological Society in Atlantic City a paper by Tucker and Hellwig integrating this anatomic finding with clinical findings. I believe from that moment history was made.

The work of Hill and his associates, as presented at this meeting and their scientific exhibit are a confirmation of the work of Johnson. This work is a classic contribution to proctologic literature.

From now forward consideration of clinical disease of the anorectal area must include an evaluation of the anal ducts and glands. I believe that history was made in proctology this morning.

Dr. Malcolm R. Hill, Los Angeles: Dr. Tucker confined his discussion to the anal crypts and ducts. To us, in our investigation, on two occasions, anal crypts and ducts appeared to carry with them contiguous gland elements which in many instances gave evidence of secretory function which was demonstrable on proper staining.

Dr. Nesselrod's study, which has been confined to pathologic material, is interesting. I have tried to save representative tissue in operative cases to pass on to the pathologist. Proctologic sampling of the anal canal in light of proper conservation of normal continuity of tissues does not give the complete clinical pathologic picture. Dr. Nesselrod has linked the causation of hemorrhoids with infection of these microscopic elements.

HISTOPATHOLOGIC OBSERVATIONS IN A FATAL CASE OF Q FEVER

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BETHESDA, MD.

ONLY 6 fatal cases of Q fever are on record.[1] Autopsies were performed in 4 of the 6 cases; 2 were reported in the "Annual Report on the Health and Medical Services of the State of Queensland, Australia" [1a,b] 1 by Lillie, Perrin and Armstrong [1c] and 1 by Brown, Knight and Jellison.[1e] The purpose of the present report is to describe the histopathologic observations in the last of these cases.[1e] The clinical and gross postmortem findings and the procedures by which the virus was isolated have been presented in detail by Brown and co-workers [1e]

REPORT OF A CASE

A 43 year old white man died after an illness of fifteen days. During the first week, the symptoms were those of a "head cold," and the patient was able to work at his regular occupation as a cattle handler in a stockyard. During the second week he became acutely ill. Prominent clinical findings were cough, nasal congestion, chills, profuse perspiration, frontal headaches and mental confusion. The temperature was elevated, varying between 103 and 106 F. Q fever was not suspected, and a diagnosis of influenza complicated by pneumonia was made. Therapy included the administration of penicillin in oil, sulfadiazine and oxygen, and was ineffectual. An examination of the blood three days before death produced normal values for erythrocytes, hemoglobin and color index. However, there was slight leukopenia; the total white blood corpuscle count was 5,000 per cubic millimeter, and the differential count was 74 neutrophilic granulocytes, 25 lymphocytes and 1 eosinophilic granulocyte per hundred cells. The patient died suddenly after a convulsive seizure.

Gross Postmortem Findings (forty hours after death).—The body had been embalmed by arterial injection only. Pertinent findings were limited to the thorax. Excess fluid was noted in the pleural and pericardial cavities, and all the lung tissue appeared to be consolidated except for a small area in the upper lobe of the right lung. The cut surfaces of the lung tissue were wet, and

From the Pathology Laboratory, Experimental Biology & Medicine Institute, National Institutes of Health, Bethesda, Md.

1. *(a)* Annual Report on the Health and Medical Services of the State of Queensland, 1936-1937, vol. *22*; *(b)* 1938-1939, vol. 52. *(c)* Lillie, R. D.; Perrin, T. L., and Armstrong, C.: Pub. Health Rep. **56**:149, 1941. *(d)* Irons, J. V., and Hooper, J. M.: J. A. M. A. **133**:815, 1947. *(e)* Brown, D. C.; Knight, L. A., and Jellison, W. L.: California Med. **69**:200, 1948.

no purulent material was seen. A firm fibrinous clot, about 8 cm. in length, was found in the branch of the pulmonary artery leading to the upper lobe of the left lung. The gross postmortem diagnoses were atypical pneumonia and pulmonary embolism.

Isolation of Virus.—In guinea pigs inoculated with blood drawn three hours after death and with sternal marrow obtained at autopsy symptoms consistent with Q fever developed, and complement fixation tests of their serums were positive for Q fever in dilution of 1:64 or greater. Rickettsias were demonstrated in smears from some of the inoculated animals.

Microscopic Observations.—Heart: Sections of the anterior wall of the left ventricle revealed marked patchy fibrosis of the myocardium and moderate to marked arteriosclerosis of the anterior descending branch of the left coronary artery. The lumen of one large collateral of the descending branch was completely obliterated by an old, organized thrombus. In a section from a localized area of thinning near the apex of the left ventricle the muscle fibers had been almost completely replaced by dense fibrous tissue, which contained scattered small accumulations of hemosiderin.

Lungs: Sections from the areas of gross consolidation revealed a confluent pneumonic process. The exudate was moderately abundant in many areas, but in scattered foci it was scanty. Frankly hemorrhagic exudate was encountered in some alveoli, but for the most part erythrocytes were inconspicuous, and numerous large mononuclear cells were intermingled with fewer neutrophilic granulocytes and occasional lymphocytes. Some of the large mononuclear cells were of the macrophage type and were actively phagocytic, while others exhibited large round to oval leptochromatic and trachychromatic nuclei and relatively scanty basophilic cytoplasm. Large mononuclear cells of the latter type often lined portions of interalveolar septums. A loose fibrin network was often encountered in the exudate, and occasionally the fibrin was condensed at the periphery of an alveolus or an alveolar duct, forming an indistinct hyaline membrane. Interalveolar septums were focally thickened and irregularly congested, and hyaline thrombi were sometimes seen in septal capillaries. The small bronchi and bronchioles contained exudate similar to that seen in the alveoli, and partial desquamation of mucosal epithelium was occasionally noted. In a few bronchi portions of denuded mucosa were covered by a single layer of flattened, deeply stained epithelial cells. Medium and large bronchi seldom contained exudate and were not involved by the inflammatory process. Small numbers of large mononuclear cells and lymphocytes were seen in peribronchial and perivascular connective tissue and were present in moderate numbers focally in the pleura and in edematous interlobular septums.

Sections from areas of the lung which were least involved grossly showed moderate congestion, patchy edema, focal hemorrhage, and small to moderate numbers of hemosiderin-laden macrophages in many alveoli. Scattered small pneumonic areas were present, and although neutrophilic granulocytes predominated in many of these foci, large mononuclear cells and lymphocytes were also present, and the large mononuclear cells were often numerous. Lymphocyte and mononuclear cell infiltration of peribronchial and perivascular connective tissue was more marked than that occurring in the areas of confluent pneumonia and was often associated with similar infiltration of thickened interalveolar septums.

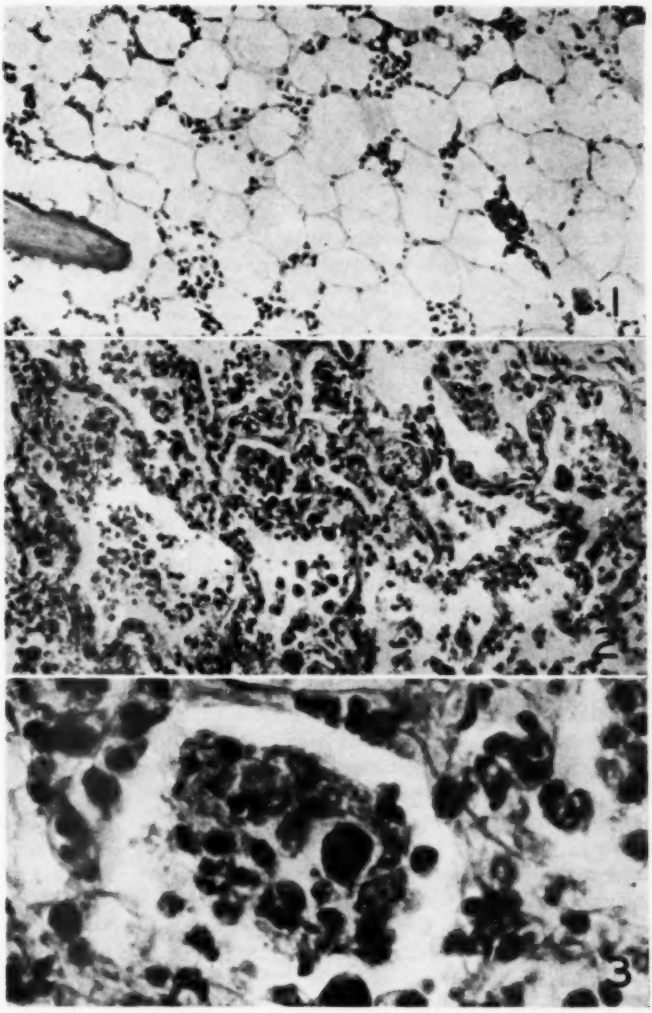

Fig. 1.—Area of hypoplasia in sternal marrow. × 225.

Fig. 2.—Thickening of interalveolar septums. Note alveolar exudate showing predominance of mononuclear cells. × 225.

Fig. 3.—Swollen alveolar lining cells. Note mononuclear cells in the alveolar exudate. × 860.

A careful search through several sections stained with the azure-eosin technic [2] and Gram's stain failed to reveal rickettsias or other micro-organisms.

Sections of blood clot taken from a branch of the pulmonary artery leading to the upper lobe of the left lung were consistent in appearance with postmortem blood clot.

Liver: The organ revealed no abnormality.

Spleen: Plasmacytes and large mononuclear cells were found in slightly increased numbers in the red pulp.

Lymph Nodes: A peribronchial lymph node exhibited considerable deposition of carbon. In a mesenteric lymph node, widely dilated sinuses contained a moderate number of large mononuclear cells.

Kidneys: There was slight to moderate fibrous intimal thickening of the small and medium-sized renal arteries.

Adrenal Glands: The adrenal glands showed no abnormality.

Sternal Marrow: In a few patchy areas the marrow was almost, if not completely, devoid of myeloid cells. In these areas a little precipitated albuminous fluid was sometimes seen, and occasional small accumulations of hemosiderin. The remaining, major portion of the marrow revealed the normal proportion of myeloid cells and fat. In the cellular areas, erythropoiesis was within normal limits, and megakaryocytes were present in usual numbers. However, mature granulocytes were relatively few, and the majority of cells were classed as myelocytes and metamyelocytes.

The histopathologic diagnoses were: bronchopneumonia; focal hypoplasia of bone marrow with a moderate left shift of cells of the granulocytic series; moderate coronary arteriosclerosis, with an old, organized occlusive thrombus of a major collateral of the left anterior descending branch of the coronary artery; old, healed infarction of the anterior wall of the left ventricle; slight renal arteriosclerosis; congestion of spleen.

COMMENT

In the case now described there is some evidence to suggest that the bronchopneumonia and the hypoplasia of marrow may have been due to the direct action of the causative agent of Q fever. The lesions of other organs were considered to be either nonspecific or degenerative in type. The Q fever virus was isolated from blood and marrow and satisfactorily identified, and bacteria could not be demonstrated in appropriately stained sections of lung tissue. The bronchopneumonia with mononuclear cells predominating in the exudate was consistent in type with a rickettsial infection and resembled the bronchopneumonia in another fatal case of Q fever.[1c] In the other 2 cases of Q fever in which autopsies were made,[1a, b] histopathologic observations were not recorded, and a comparison of lesions cannot be made. In experimental Q fever infections of mice,[3] guinea pigs [4] and monkeys [1c]

2. Lillie, R. D.: Histopathologic Technic, Philadelphia, The Blakiston Company, 1948, p. 82.

3. Perrin, T. L., and Bengston, I. A.: Pub. Health Rep. **57**:790, 1942.

4. Lillie, R. D.: Pub. Health Rep. **57**:296, 1942.

bronchopneumonia was frequent and, as noted in 2 human cases, mononuclear cells predominated in the exudate. However, in the experimental infections there were lesions in many other organs and tissues. Foci of hypoplastic marrow were found only in the human case described in this report and in the experimentally infected mice. Rickettsias were not demonstrated in tissue sections from any of the human patients, nor in those from the monkeys or the guinea pigs. They were seen in sections from the mice only after several serial mouse to mouse passages of the virus.

The available evidence is insufficient definitely to implicate the virus of Q fever as the etiologic agent responsible for the major pathologic changes in the case here presented. Many additional cases must come to autopsy and be carefully studied before the pathology of human Q fever can be established. In such cases, adequate bacteriologic examinations should supplement and control virus isolation procedures because only limited reliance can be placed on the demonstration that microorganisms are or are not present in tissue sections, and rickettsial lesions cannot be differentiated from certain bacterial lesions on the basis of histopathology alone.

As a final point of interest, attention is directed to the fact that important nonrickettsial lesions were encountered in 3 of the fatal human cases in which autopsies were made. A complete record of the postmortem observations was not included in the report of the fourth case.[1b] In 1 of the 3 cases widespread bilateral pulmonary tuberculosis was observed,[1a] in another cardiac enlargement and dilatation with a widened mitral orifice,[1c] and in the present case marked coronary arteriosclerosis with an old, organized myocardial infarct. It is suggested that these lesions probably exerted an unfavorable influence on the course of the disease. This is in accord with the clinical observation that deaths from Q fever are rare in otherwise healthy persons.

SUMMARY

The sixth recorded fatal human case of Q fever is that of a 43 year old man who died after an influenza-like illness lasting fifteen days. Significant lesions encountered on histopathologic examination included bronchopneumonia characterized by a predominantly mononuclear cell exudate, focal hypoplasia of the bone marrow with a moderate left shift of cells of the granulocytic series, and moderate to marked coronary arteriosclerosis with an old, organized myocardial infarct. When the lesions were compared with those reported in other fatal human cases and with experimental infections it appeared that the bronchopneumonia and the lesions of the marrow may have been due to the infectious agent of Q fever. It is suggested that the cardiac condition may have exerted an unfavorable influence on the course of the disease.

THE MOLE AS A POSSIBLE RESERVOIR OF POLIOMYELITIS

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ST. LOUIS

IF A SINGLE animal or insect is ever found to be the reservoir of poliomyelitis, serving as a host or a vector, one may anticipate (1) that it will be found throughout the temperate and tropical zones; (2) that it will account for the frequently observed tendency of the disease to start and to have a higher incidence in rural than in urban populations; (3) that it will account for the seasonal tendency of the disease in man, and (4) that it will be so inconspicuous as to have escaped consideration to date. Numerous animals have been considered, many of them being more or less susceptible to the virus after adaptation; yet none so far has merited serious consideration as the natural reservoir, and only in the simians has the disease been observed to occur naturally. After considering the fossorial habits and the geographic distribution of the ground mole one is struck with the possibility of this animal fulfilling the four anticipated features of a natural host for poliomyelitis.

The mole is found in practically every country in the temperate and tropical zones, even being found in Siberia.[1] Only islands such as Ireland [2] and islands of the Pacific Ocean such as the Hawaiian group,[3] which have arisen geologically separated from the continental mainlands, are free from moles.

It is obvious that a higher percentage of the rural than of the urban population comes in contact with these animals; yet the prevalence of moles in the city parks offers urban dwellers sufficient contact with them—disregarding trips to the country. Furthermore, spot maps of urban epidemics of poliomyelitis frequently show a centripetal spread.

That moles come out on the surface of the ground during the hot summer months in search of worms and water is a well established fact among naturalists,[2] and even the untrained naturalist is impressed by the observation that in hot weather their runways are so shallow that they are covered merely by the roots of overlying vegetation. Coupled with these observations is the even more important one that during hot weather people lie on the ground, frequently in close contact

From the Department of Anatomy, Washington University.

1. Skalon, W. N.: Zool. Anz. **77**:307, 1928.
2. MacDougall, R. S.: Tr. Highland Agric. Soc. Scotland **14**:80, 1942.
3. Hamre, C. J.: Personal communication to the author.

with mole runs. Furthermore, in cold weather moles use their deeper runs and refuse to burrow new runways when the ground is frozen. In fact, the mole is vermivorous, and the depth of his burrow will vary seasonally with the level at which his food, chiefly the earthworm, is to be found. Earthworms breed in the hot summer months and are known to come to the surface and draw leaves into their burrows for nesting purposes. The mole is voracious; with it eating is said to be a frenzy, and it will incur many dangers to get food.

Numerous animals and many insects have been studied as a possible reservoir for the virus of poliomyelitis, but as yet the mole has escaped consideration. This is probably due to the fact that his subterranean existence leaves him somewhat inconspicuous. However, this same subterranean existence could easily facilitate an inherently inconspicuous harboring of the virus between the seasons of common epidemic occurrence of the disease in man. Were the animal to be found susceptible to the virus one could easily conceive of epizootics occurring undetected by man. If the disease were found to be highly fatal to them, they would die and bury the virus with them, only to pass it on to another mole when the latter eventually broke into and took over the run left idle by the dead mole. It is known that the virus of poliomyelitis when kept in a moist state and in darkness will remain viable at room temperature, for periods varying from 25 to 114 days.[4] In the natural environment of the mole, the requirements of darkness and moisture would be maintained, and the temperature probably would be lower than room temperature. Furthermore, if the disease should be less fatal among moles and leave surviving but partially paralyzed animals, one would have to consider whether there are carrier states, how long the virus is discharged in the stools and pharyngeal secretions and whether dissemination occurs during the mating season (March and April).

It is now well established that the virus of poliomyelitis can be recovered from the pharyngeal secretions for a few days and from the stools for several weeks following the onset of human infection. Also the virus has been demonstrated in sewage coming from epidemic areas on at least eight occasions.[5] Should the mole be found susceptible to the virus, the possibility of an epizootic starting with exposure to human excreta is likely.

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4. Landsteiner, K., and Levaditi, C.: Ann. Inst. Pasteur **24**:833, 1910. Kling, C.; Levaditi, C., and Lepine, P.: Bull. Acad. de méd., Paris **102**:158, 1929.

5. (*a*) Paul, J. R., and Trask, J. D.: Am. J. Pub. Health **32**:233, 1942. (*b*) Toomey, J. A.; Takacs, W. S., and Weaver, H. M.: Am. J. Dis. Child. **70**:293, 1945.

In any event one is faced with the irrefutable fact that man and mole come much closer to each other during the hot summer months, and it seemed advisable to investigate the mole's susceptibility to the virus of poliomyelitis.

MATERIALS AND METHODS

All moles were caught manually in Forest Park in St. Louis. An attempt to keep a stock bin of animals was soon abandoned because of the tendency of moles to fight until there is one surviving victor when more than one is placed in the same container. Consequently each mole was kept in an individual metal can that was partially filled with dirt. After moles 6, 7, 8 and 9 became paralyzed, probably from being exposed to contaminated dirt, new dirt was used for each animal, and used dirt was decontaminated with cresol solution before being discarded. A container of water was kept countersunk to the level of the surface of the dirt. All moles except mole 36 were fed diced horse meat or ground beef or both. Mole 36 was fed solely on earthworms. Feeding was effected by merely placing the meat on the surface of the dirt. Rectal temperatures were taken daily after inoculation or exposure and more frequently if the animal was being observed while showing symptoms. The isolation technic was employed in handling the animals, the hands and arms being washed with soap and water and rinsed in alcohol before handling, and between, moles. In addition, the cotton glove worn on the hand used to hold the mole while its temperature was being taken was boiled from 5 to 10 minutes between moles.

The experiment was begun by inoculating moles 1 and 2 intracerebrally with a filtrate of a mouse brain infected by a rodent-adapted strain of Lansing virus. A second but similarly infected mouse brain was the source of the inoculums used for moles 3, 4 and 5; a third similar mouse brain was used as the source of inoculums for moles 33, 34, 35 and 36. The human brain and spinal cord used as inoculum for the animals indicated in table 1 were obtained at the autopsy of an 11 year old boy who died in a respirator in the summer of 1946 with a classic clinical and pathologic picture of poliomyelitis. This virus had been preserved in water and glycerin at ordinary ice box temperature, and the particular strain of virus involved is unknown.

Stools from 4 children currently stricken with poliomyelitis (summer of 1948) served as the source of inoculums or of soil contamination for moles 37 to 43, inclusive. Clinically these children showed paralysis and an abnormality of the spinal fluid consistent with poliomyelitis.

Each inoculation passage from mole to mole, from mole to Swiss mouse or cotton rat, and between mouse and rat was made with a saline dilution (approximately 1:10 to 1:20) of a filtrate from wet brain and spinal cord. All filtrates were made by passing the material through a Chamberland L-5 filter except the stool filtrates injected into moles 40, 41, 42 and 43, for which the stools were passed through a Berkefeld N candle. The filtrates of the latter were in 1:1 dilution.

Prior to inoculation moles 24 through 29, inclusive, were subjected to a period of starvation varying from 24 to 27 hours with the hope that the hypoglycemic state might enhance their susceptibility to the virus as Sandler[6] found it did for rabbits.

No animals were killed, all being observed until they died. Autopsies were performed as soon after death as practicable. In many instances moles died during

6. Sandler, B. P.: Am. J. Path. **17**:69, 1941.

the night, and in other instances the animals were placed under refrigeration for varying periods. Brain and spinal cord saved for subsequent passage were preserved in the fresh state in dry ice (solid carbon dioxide). Tissues studied microscopically were fixed in acetic acid–Zenker's solution and stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

Two Types of Terminal Picture.—One type of terminal picture encountered, and the one most classically representing a poliomyelitic attack, was that of massive paralysis of the abductor group of muscles of the forelegs. In its most complete form there was no motion of the anterior extremities, and the animals invariably were found on the surface, usually on their backs, unable to turn over. They frequently would lie in this position for 24 to 36 hours with the forelegs inactive while the hindlegs kept up a continuous running motion. When these animals were turned over to a normal running position, their forelegs were adducted, motionless, resembling sled runners as the hindlegs frantically propelled the body. This type of activity continued until death. Starvation and dehydration are undoubtedly important factors in the failure of these animals to recover.

Slight variations of this picture were noted. In some cases only one foreleg demonstrated paralysis. This resulted in the animal's turning in circles, with the affected side serving as the axis. In others there was transient paralysis, with fairly good function returning later. In the case of mole 10, at one time the hindlegs were paralyzed, and propulsion was effected by use of the forelegs. Paralysis of the hindlegs was definitely observed in 4 cases and questionably in another, compared with 13 definite and 3 questionable cases of involvement of the forelegs. Animals judged to show questionable involvement of the forelegs were so classified because they made frantic attempts to dig into the dirt when disturbed but did not appear to have the strength to do so.

The other type of terminal picture observed was that in which the animal stayed on top of the dirt, "huddled up," with its neck flexed and its head held against its chest. Animals so affected frequently showed instability of equilibrium, falling from side to side. If sufficiently stimulated, they would move around, frequently burrowing into the dirt, only to return to the surface in a few minutes to resume their former position. Definite ability to move the extremities was demonstrable. Respirations were usually labored at first and shallow terminally. Unfortunately, the mole seems normally to employ chiefly an abdominal type of respiration. The picture closely resembles what has been previously referred to as a vagal type of poliomyelitis in experimental animals.

In 1916 Rosenau and Havens[7] described a fulminating type of death observed in rabbits inoculated with poliomyelitis virus. The symptoms were explosive in character, and death usually occurred within a few hours to 2 days. The death of mole 19 in this series fits this picture, and a similar type of death may have occurred in moles dying during the night.

Survival of Animals.—No animals were killed, and of the 43 moles used in the experiment only 2 still survive (moles 40 and 43). Several of the moles may have died as the result of inoculation trauma or other causes; this is especially true of moles 24, 25, 27, 28 and 29, which were subjected to a period of starvation prior to and subsequent to inoculation. It is doubtful if the mole can survive more than 36 hours of starvation. All animals have been included in table 1 for completeness.

7. Rosenau, M. J., and Havens, L. C.: J. Exper. Med. **23**:461, 1916.

TABLE 1.—*Data on Moles Undergoing Experiments*

Mole	Days in Captivity Before Inoculation	Route of Inoculation *	Volume of Inoculum (Cc.); Amount of Stools (Gm.)	Source of Inoculum †	Days from Inoculation to Death	Weight at Autopsy, Gm.	Lethargy	Anorexia	Paralysis of Forelegs	Excitability	Urinary Incontinence	Terminal Hypopyrexia	Paralysis of Hindlegs	Pulmonary Edema, Autopsy	Intestinal Hemorrhage
1	10	I. C.	0.1	Mouse brain	5	102	..	+	..	..	..	..	..	+	..
2	10	I. C.	0.1	Mouse brain	34	104	..	..	+	..	..	..	..	..	..
3	1	I. C.	0.06	Mouse brain	106	73	+	..	+	+	+	..	+	..	..
4	1	I. C.	0.06	Mouse brain	30	93	..	..	..	..	..	..	..	..	..
5‡	1	I. C.	0.06	Mouse brain	3	..	..	..	..	..	..	..	..	..	..
6	14	Spon.	...	Contamination	?	68	+	+	+	..	..	..	..	+	..
7	?	Spon.	...	Contamination	?	118	+	+	+	+	..	..	..	+	..
8	1	Spon.	...	Dirt mole 7	2	64	..	+	..	..	..	..	..	+	..
9	21	Spon.	...	Dirt moles 7 and 8	8	127	+	+	+	+	..	+	?	+	..
10	4	I. C.	0.1	C. N. S. mole 2	14	81	+	+	+	+	..	..	+	+	..
11	45	Spon.	...	Dirt moles 7, 8 and 9	23	56	+	+	..	..	+	+	..	..	..
12	20	I. C.	0.1	Human C. N. S.	28	97	+	+	+	+	..	+	+	+	+
13	19	I. C.	0.1	Human C. N. S.	96	66	+	+	+	+	..	+	..	..	..
14	1	I. C.	0.1	C. N. S. mole 9	8	66	+	..	..	+	..	..	..	+	+
15	1	I. C.	0.1	C. N. S. mole 9	8	73	+	+	..	..	+	+	..	..	+
16	39	Spon.	...	Dirt mole 14	27	63	+	+	?	..	+	+	..	+	..
17	27	Spon.	...	Dirt mole 15	20	56	+	+	+	+	+	+	+	+	+
18	31	I. C.	0.1	C. N. S. mole 15	70	85	..	+	+	+	..	..	..	+	+
19	30	I. C.	0.1	C. N. S. mole 14	4	71	+	+	+	..	+	..	..	..	+
20	8	Spon.	...	Exposure ?	<10	63	+	+	..	..	..	..	..	..	..
21	9	I. C.	0.1	C. N. S. mole 19	47	96	+	+	?	..	..	..	..	+	..
22	4	I. C.	0.1	C. N. S. mole 12	39	72	..	+	?	+	..	..	..	+	+
23	41	I. C.	0.1	C. N. S. mole 10	80	55	..	+	..	..	+	..	..	+	..
24	1	S. C.	1.0	Human C. N. S.	2	92	..	..	..	+	..	..	..	+	..
25	1	I. V.	0.3	Human C. N. S.	<1	108	+	+	..	..	..	..	..	+	..
26	1	I. C.	0.1	Human C. N. S.	124	44	+	+	..	..	+	..	..	+	..
27	1	I. C.	0.1	Human C. N. S.	<1	73	..	..	..	..	..	..	..	+	..
28	1	I. N.	0.5	Human C. N. S.	62	79	..	..	..	..	..	..	..	+	..
29	3	I. N.	0.5	Human C. N. S.	18	80	..	..	..	..	..	..	..	..	+
30	54	I. V.	0.3	Human C. N. S.	139	73	..	?	..	..	..	+	..	..	+
31	8	I. C.	0.1	C. N. S. mole 22	2	89	..	..	..	..	..	..	..	+	+
32	1	I. C.	0.1	C. N. S. mole 21	54	56	+	+	+	..	+	..	..	..	+
33	3	I. C.	0.1	Mouse brain	30	60	..	..	..	+	..	..	..	+	..
34	4	I. C.	0.1	Mouse brain	2	62	+	..	+	..	..	+	..	..	+
35	3	I. C.	0.1	Mouse brain	1	83	+	+	..	..	..	..	..	..	+
36	1	I. C.	0.1	Mouse brain	4	60	+	+	..	..	..	..	..	..	+
37	65	Exp.	20 Gm.	Human stool	31	103	..	+	..	..	+	..	..	..	..
38	56	Exp.	20 Gm.	Human stool	9	89	..	..	..	..	..	..	..	..	+
39	3	Exp.	8 Gm.	Human stool	27	68	+	+	..	..	+	+	..	..	+
40§	7	I. C.	0.1	Human stool	..	..	..	..	..	..	..	..	..	..	..
41	7	I. C.	0.1	Human stool	22	55	..	..	..	..	+	..	..	+	..
42	5	I. C.	0.1	Human stool	5	59	+	..	..	..	..	..	..	..	..
43§	4	I. C.	0.1	Human stool	..	..	..	..	..	..	..	..	..	..	..

* I. C. means intracerebrally; Spon., spontaneously; S. C., subcutaneously; I. V., intravenously; I. N., intranasally; Exp., exposed.
† C. N. S. means central nervous system.
‡ No autopsy was made.
§ The mole was alive still at the end of the experiment.

The time of survival is variable and unpredictable, regardless of the route of inoculation or the source of the inoculum or the relative position of the animal in series passage. Animals have died in from 98 hours to 139 days and still demonstrated convincing pictures of one or the other of the two types of pictures seen terminally.

The most reliable results were found in the animals which were inoculated intracerebrally or which were exposed to feces from a human patient who had poliomyelitis or to dirt previously contaminated by an infected animal. For the latter group (moles 6, 7, 8, 9, 10 and possibly 20) the survival time varied from 2 to 23 days of known exposure. All of these animals terminally demonstrated to a convincing degree either paralysis of the extremities or an overwhelming involvement of the so-called vagal type.

The series of animals inoculated by other routes (intranasal, intravenous and subcutaneous) is too small and the survival times and other results too equivocal to warrant conclusions. All such routes, and in addition the intraperitoneal route, should be investigated more thoroughly.

Miscellaneous Findings or Observations.—For a mole to come to the surface is abnormal behavior, as they are wary animals and come to the surface only to drink when the laboratory is quiet. Of the 41 moles to die to date in this experiment, only 6 have died beneath the surface of the dirt.

Anorexia, as judged by the leaving of meat on the surface, was noted in 26 moles. Lethargy was noted in 23 and, of course, could not be observed in animals undergoing a fulminating type of death during the night. Paralysis of extremities already has been discussed. Excitability was manifested in several ways; many moles came to the surface and ran around for hours—mole 17 ran backward; others showed hyperactivity on casual stimulation; the constant running motion of the hindlegs, with the mole on its back, was obviously the result of cortical stimulation.

Fourteen moles remained for a considerable time on top of the dirt; others found dead on the surface in the morning undoubtedly were showing this abnormal behavior for some time before death. Hyperpyrexia was detected only nine times but undoubtedly would have been found more frequently had temperatures been taken more often. Unfortunately, moles are difficult to keep under laboratory conditions, and it seemed advisable to minimize their handling. Rectal temperatures of 37.5 to 38.5 C. (99.5 to 101.3 F.) were regarded as hyperpyrexia. This elevation of temperature frequently antedated any signs of lethargy or paralysis. Terminal hypopyrexia was encountered 10 times, and probably all animals would exhibit this feature terminally were more frequent temperatures taken. Rectal temperatures below 34.5 C. (94.1 F.) were considered as hypopyrexia.

Cardiac arrhythmia was observed in the course of taking temperatures of moles 9 and 42. In the former it consisted of the dropping of every fourth beat; in the latter it consisted of frequent extrasystoles. This feature should be given more thorough investigation, especially in animals showing a terminal picture suggesting overwhelming vagal involvement.

The remaining symptoms enumerated in table 1 are self explanatory. Fleas, and occasionally mites, were observed in the fur of 9 animals. The percentage of moles so infested is undoubtedly much higher than this would indicate, as the parasites readily leave their host when the body becomes cold and, consequently, were not found on moles dying during the night or those placed in the ice box prior to autopsy. Their presence is noted here because they may conceivably play a role in the dissemination of disease herein referred to as probably due to exposure to contaminated dirt.

The enumeration of cases in which pulmonary edema and intestinal hemorrhage occurred is based solely on gross observations. The incidence of these pathologic findings would probably be increased by microscopic examination.

Pathologic Observations.—Neuronophagia and inflammatory cell reaction in the meninges or perivascular spaces were entirely lacking in all moles and in the Swiss mice and cotton rats in which passage was effected. Practically all animals showed some degree of vascular engorgement. In most animals there were profound degenerative changes of the cells of the anterior horns of the spinal cord and, to a lesser degree, of the nerve cells elsewhere in the spinal cord, the brain stem, the cerebellar nuclei and the cerebral cortex. The earliest phase of this change is a dissolution of the Nissl substance, followed by a pale, powder blue staining of the cytoplasm, an indistinctness of the cytoplasmic membrane, vesicularity and chromatolysis of the nucleus and eventually an almost complete ablation of the entire nerve cell. By correlating the degree of changes with the maximum possible postmortem time before fixation and carefully studying control material left at room and ice box temperatures for known periods before fixation one became convinced that the changes were autolytic rather than cytolytic.

TABLE 2.—*Observations on Control Moles*

Control mole	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Days in captivity before being used	0	0	1	3	2	0	0	1	25	0	0	100	47	4	4
Hours in ice box post mortem before fixation	0	9	0	23	24	0	0	0	0	4	4	1.5	3	0	4
Hours at room temperature post mortem before fixation	0	0	0	0	0	24	16	8	4	5	5	0	0	4	18
Inflammatory meningeal reaction	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0
Degree of autolysis of anterior horn cells *	1	1	0	2	3	4	4	4	3	3	3	1	1	2	4

* 0 means no autolysis; 1, dissolution of Nissl substance and indefiniteness of the cytoplasmic membrane in a few cells; 2, chromatolysis and lysis of Nissl substance affecting one third to one half of the cells; 3, same as 2, except that one half to two thirds of the cells were involved; 4, autolytic changes in all cells of the anterior horns.

In 6 moles of the experimental series (6, 15, 16, 22, 23 and 25) petechial hemorrhages were noted at various sites throughout the central nervous tissue.

Control Observations.—Fifteen moles were used as various types of controls as indicated in table 2. Of these controls, moles 1 to 3 were examined immediately after their deaths, and others were left at room or ice box temperatures for varying periods, to ascertain whether the changes found in the anterior horn cells of inoculated or exposed moles were the result of cytolysis or autolysis. A careful comparison of the changes found in the control and the experimental series leaves no doubt that they are autolytic in character.

The lack of inflammatory cell reaction in the central nervous tissues of the experimental animals and the impression of a paucity of leukocytes in the vascular channels raised the question whether the mole was capable of mobilizing leukocytes in response to an inflammatory stimulus. Control moles 12 and 13 were inoculated intracerebrally with a saline suspension of fresh blood agar subculture of Pneumococcus type I. Microscopic sections of this material revealed a definite ability to respond with polymorphonuclear leukocytes.

Control moles 14 and 15 were studied to rule out several theoretic possibilities. In all respects these 2 animals were fed and handled as were the animals of the experimental series except that they were isolated two floors distant from the

other experimental animals. First, it seemed advisable to check on a possible avitaminosis resulting from the artificial diet of captivity; second, it was conceivable that the trauma of daily handling and temperature taking might lead to a bizarre heretofore undescribed illness, and third, it seemed advisable to rule out the possibility of the animals reacting to the inoculation of foreign proteins. These 2 animals were inoculated both intracerebrally and intravenously with brain and spinal cord from 2 moles which were killed almost instantly by a fracture of the spinal column when caught. Control mole 15 died after 36 days, and autopsy revealed extensive hemorrhagic pneumonia and peritonitis. Control mole 14 expired 101 days after inoculation; autopsy revealed acute trichiniasis. Therefore, it does not seem likely that death and the symptoms so consistently found in the experimental group could be due to dietary deficiency, to trauma of daily handling or to a foreign protein reaction. Furthermore, many animals have been kept as stock moles for periods of 30 to 100 days with no evidence of dietary deficiency.

Passage from Moles to Swiss Mice and Cotton Rats.—Each intracerebral passage was made with a 1:10 saline dilution of a filtrate of wet mole brain and spinal cord. The filtrates were obtained by light centrifugation of the material and passage through a Chamberland L-5 filter. Subsequent mouse and cotton rat passages were made with diluted filtrates of material similarly treated. Mice received 0.03 cc. and cotton rats 0.05 cc. of diluted filtrate. In all 39 Swiss mice and 35 cotton rats were so inoculated, either directly with mole tissue filtrate or with subsequent mouse or cotton rat tissue filtrate. Of these, 27 Swiss mice and 17 cotton rats still survived after periods varying from 15 to 69 days. Moles 2, 3, 9 and 21 were used as sources of the inoculums of four series. Infectivity has been demonstrated through three serial passages from moles 2 and 9, and through but one passage from moles 3 and 21. However, the original passage of material from mole 9 resulted in 1 cotton rat dying in 17 days, from which subsequent passage was successful, while 4 mice and 2 more cotton rats succumbed within 5 days of each other 60 to 65 days after injection.

As in the experimental mole series, no neuronophagia, perivascular lymphocytic cuffing or meningeal inflammatory cell reaction was found on microscopic examination of tissues of the central nervous system.

Failure to Effect Passage to Monkeys.—Intracerebral inoculation of 0.25 cc. of a 1:10 saline dilution of the filtered wet brain and spinal cord of mole 19 failed to produce paralysis or untoward symptoms in an old rhesus monkey during the course of 120 days of observation. As nothing was known of the past history of the monkey other than that she had been used for the production of anti-Rh serum, it was decided to use recently imported young rhesus monkeys and larger doses, comparable to the doses used by Marks.[8] Accordingly, on July 28, 1948, 2.0 cc. of a 1:10 saline dilution of the filtered wet brain and spinal cord of mole 38 was injected intracerebrally into a young rhesus monkey. (The filtrate had been obtained by passing the material through a Berkefeld N candle.) On July 30 the same amount of inoculum, similarly prepared, from mole 42 was injected intracerebrally into another recently imported young rhesus monkey. Neither of these animals has shown paralysis or any untoward symptoms after 220 to 222 days of observation.

COMMENT

This report is destined to be controversial, as lesions typical of poliomyelitis were lacking. The lack is by no means unique in the mole,

8. Marks, H. K.: J. Exper. Med. **14**:116, 1911.

similar results having been reported by various investigators attempting to transmit poliomyelitis to other animals.

Marks [8] reported convulsions and death observed in rabbits after inoculation of poliomyelitis virus, yet found no lesions comparable to those observed in man and monkey in the tissues of the central nervous system. He did, however, succeed in passing the virus from 3 rabbits to 3 monkeys by massive intracerebral inoculation (4.0 cc., 3.0 cc. and 2.5 cc.) and demonstrated classic lesions in nerve tissue. Marks's rabbits underwent no paralysis but succumbed after convulsive seizures.

Rosenau and Havens [7] reported that 22 of 54 rabbits died, after being inoculated with poliomyelitis virus. Death was preceded by one or the other of two terminal syndromes similar to those exhibited by moles in this series. They also were unable to fix their virus on passage, and the lesions of the central nervous system were quite dissimilar to those observed in man and monkey. The degeneration of large motor cells reported by them may be essentially autolytic in character, as no controls were employed for comparison. They succeeded in passing the disease-producing agent to a monkey, which died of a respiratory rather than a typical paralytic syndrome. The pathologic condition observed in the monkey resembled that in the rabbit and was far short of a true duplication of the usual human or simian lesions. A quotation from their article is pertinent at this point:

> . . . If the virus of poliomyelitis may be so altered in the rabbit as scarcely to be recognizable, may it not be still more profoundly changed in other animals? The conjecture then arises that poliomyelitis, instead of being limited naturally to man and experimentally to monkeys, may in fact occur in other animals in unnoticed or unrecognized form."

Sandler [6] also adapted the rabbit to poliomyelitis virus by first inducing hypoglycemia by starvation and/or insulin. Pathologically he found no neuronophagia or perivascular or meningeal cellular infiltration. He described intracellular changes which might represent autolysis. He was able to produce typical lesions in the monkey by inoculating the infected rabbit material. Paralysis was evident in only 2 of 11 animals.

Hassin [9] concluded that the chief pathologic changes found in the rabbit are of a cellular degenerative rather than of a cellular infiltrative or neuronophagic type. In paralysis produced in sheep and believed by Hassin to be poliomyelitic, the pathologic changes were not uniform but tended to simulate more closely those found in man and monkey than those produced in rabbits. Hassin's results must be interpreted with caution, however, as it is not stated whether the inoculums were filtered material; no attempt seems to have been made to differentiate between autolytic and cytolytic cellular changes, and

9. Hassin, G. B.: M. Rec. **92**:89, 1917.

in 2 sheep and 1 rabbit cerebrospinal fluid appears to have served as the infective inoculum. It is now rather generally accepted that the virus cannot be recovered from this source. He concluded, however, that the replica of the histopathologic changes observed in man and in the monkey is not found in the rabbit or the sheep.

A slight variation from the classic histopathologic picture of poliomyelitis has been reported observed in the guinea pig. In 1913 Neustaedter [10] reported that 2 guinea pigs became spontaneously paralyzed after rather undeniable exposure to a monkey ill of experimental poliomyelitis. On first passage of spinal cord from one of the guinea pigs to a second guinea pig no perivascular infiltration was found and only slight neuronophagocytosis, but on subsequent passage through other guinea pigs these features reappeared in classic form.

Dalldorf and Sickles [11] recently reported the isolation of a virus from the stools of 2 patients who had poliomyelitis, which produced paralysis in suckling mice and hamsters with an absence of lesions of the central nervous system. They described severe and widespread changes occurring in skeletal muscles. Changes described as a loss of striations, acidophilic reaction and fragmentation could be autolytic in character. However, they described intense proliferation of young muscle cells with endothelial cell phagocytosis. Their report prompted a search for similar changes in the moles herein concerned. Muscles from the abductor group of the forelegs and the diaphragm were removed from the carcasses, after having been fixed in formaldehyde solution. No such changes could be identified with certainty in any of the animals of the experimental or the control series.

Armstrong [12] showed, and others have confirmed, that the cotton rat is susceptible to the Lansing strain of poliomyelitis virus, which produces in this rat microscopic lesions in every respect similar to those observed in man and monkey. Toomey, Takacs and Weaver [5b] encountered an exception to this finding when they were identifying what appears to have been a strain of poliomyelitis virus isolated from a creek in Perryville, Ohio, in 1944. On several occasions their animals died without microscopic lesions that could be identified as those of poliomyelitis; yet they were successful in producing the classic lesions in 2 monkeys and in many of their cotton rats.

In the past critics have been reluctant to accept as poliomyelitis in animals a paralytic syndrome that fails to be accompanied by the classic lesions. It may well be that they have been too rigid in their expec-

10. Neustaedter, M.: J. A. M. A. **60**:982, 1913.
11. Dalldorf, G., and Sickles, G.: Science **108**:61, 1948.
12. Armstrong, C.: Pub. Health Rep. **54**:1719, 1939.

tations in this respect and consequently may have overlooked the natural existence of the disease in other species, as Rosenau and Havens cautioned us 32 years ago.

A second controversial point raised by the work herein reported is the possibility that an epizootic virus disease of either the mole or the mouse has been brought to the fore. The Bureau of Animal Industry of the United States Department of Agriculture [13] reports that no epizootic disease of an encephalitic type is known to occur among moles. Furthermore, the inoculum used on all occasions in this experiment was from a known poliomyelitic source. That an epizootic disease of mice might have been present in the mouse brain used is theoretically possible. However, typical lesions of poliomyelitis have been produced repeatedly by injecting this strain into Swiss mice. Were one to raise the question of the possible presence of Theiler's mouse virus one would have to admit, and explain, the reversal of the virus' tendency to produce constantly paralysis of the hindlegs to a tendency to involve the forelegs more commonly.

In addition to the four a priori concepts suggesting that the mole might be a natural reservoir of poliomyelitis, two facts have been brought out by the experiments performed to date that lend further support to such a hypothesis. First, cross infection between moles in casual contamination is apparent, and, second, the frequently encountered long survival times of some animals would greatly facilitate the subterranean harboring of the disease in epizootic form between epidemics among human beings. Were the disease to be found occurring among moles in conjunction with an epidemic among human beings, one would have a fair explanation as to why the incidence among males is somewhat greater than that among females, "for little boys are much more likely to be playing in the dirt than are little girls."

It is realized that there are many interesting and important phases of this problem that have not been investigated, and it is with sincere apologies that the work is reported with so much still to be done. It would seem to be important to learn (1) to which strains of the virus the mole is susceptible, (2) by what routes of inoculation infection is possible, (3) whether the mole flea (Paloepsylla gracilis) is a vector or possible intermediary host, (4) whether typical microscopic lesions of poliomyelitis might not be produced eventually on further serial passage in moles, Swiss mice or cotton rats, and (5) if, and for what periods, the virus might be excreted from the pharynx and in the stools. However, it seems justifiable to publish the data accumulated to date, controversial as they may be, in order that physicians may

13. United States Department of Agriculture, Bureau of Animal Industry: Personal communication to the author.

be alerted to the advisability of keeping this problem in mind in taking individual case histories, as well as to the advisability of making epidemiologic surveys of moles in conjunction with future epidemics of poliomyelitis.

The question is immediately raised whether the mole is susceptible to other virus diseases having a hot weather seasonal incidence. One thinks of rabies, eastern and western equine encephalomyelitis, and epidemic encephalitis of the St. Louis and Japanese types.

SUMMARY

Several reasons why the mole might be the natural reservoir of poliomyelitis are enumerated and discussed.

Forty-three moles have been exposed to, or inoculated with, virus of poliomyelitis obtained from the following sources: mouse brain experimentally infected with the rodent-adapted strain of Lansing virus, brain and spinal cord of a human patient who died of poliomyelitis, and stools of four infected human beings. Of these animals, but 2 have survived. Two distinctly different types of terminal behavior are described. The survival time of the infected animals is unpredictable, regardless of the source of the inoculum, the route of inoculation or the relative position of the animal in serial passage. There is no tendency of the virus to become fixed. Possible routes of inoculation other than intracerebral have not been adequately investigated.

Miscellaneous autopsy observations and terminal behavior traits are tabulated and discussed.

Successful passage of the virus from mole to mole and from mole to Swiss mouse and cotton rat is reported. Unsuccessful attempts to pass the virus to 3 monkeys is reported.

The absence of typical histopathologic lesions of poliomyelitis in the moles and the Swiss mice and cotton rats in which passage of mole brain and spinal cord was effective is discussed, and the literature concerning similar experiences of other investigators is reviewed.

Fifteen animals were employed as controls for comparative studies of autolytic changes, dietary deficiency, ability of the mole's brain to respond with mobilization of inflammatory cells, trauma of handling and reaction to foreign protein.

THE BLOOD CELLS AND THE HEMOPOIETIC AND OTHER ORGANS OF DOGS GIVEN INTRAVENOUS INJECTIONS OF 2-CHLOROETHYL VESICANTS

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IN A recent paper the quantitative changes occurring in the leukocytes of the peripheral blood and the histologic changes in the hemopoietic organs of rats poisoned with 2-chloroethyl vesicants were analyzed.[1] In the present investigation, data of a similar nature on dogs poisoned with these vesicants are presented. Better information concerning the daily changes in the blood picture has been obtained from the dogs, but because of more extensive pathologic changes, the lymph nodes and the spleen did not lend themselves to the same kind of quantitative study as was possible with those of the rats. However, it is felt that the data obtained from the poisoned dogs will supplement the information obtained from the rats and advance knowledge concerning the relations between the leukocytes of the peripheral blood and the hemopoietic organs. In addition, changes in the daily counts of the red blood corpuscles and the thrombocytes of the peripheral blood, not obtained in the rat, are included in the present paper. The data will also supplement those which have been discussed in relation to the matter of using the vesicants in tumor therapy as presented in the reports given at a symposium of the American Association for the Advancement of Science.[2]

MATERIAL AND METHODS

The material consisted of 17 male dogs which were being used by the biochemistry department of the University of Virginia under the direction of Dr. Alfred Chanutin for the study of the effects exerted by the 2-chloroethyl vesicants on the proteins and other constituents of the blood.[3] The dogs were fed a stock

From the Anatomical Laboratory, University of Virginia.

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1. Kindred, J. E.: Arch. Path. **43**:253, 1947.

2. Moulton, F. R.: Approaches to Tumor Chemotherapy, American Association for the Advancement of Science, Lancaster Pa., Science Press Printing Company, 1947, p. 95.

3. (*a*) Gjessing, E. C., and Chanutin, A.: J. Biol. Chem. **165**:413, 1946. (*b*) Chanutin, A., and Ludewig, S.: ibid. **167**:313, 1947.

laboratory diet and were kept under observation for at least a month. The animals were in good condition at the beginning of the experimental period. The vesicants used were redistilled sulfur mustard, or bis(2-chloroethyl) sulfide, dissolved in thiodiglycol (code name in this report, H), and the hydrochlorides of ethyl-bis (2-chloroethyl) amine (code name, HN-1) and tris(2-chloroethyl) amine (code name, HN-3), dissolved in isotonic sodium chloride solution just before being injected into the saphenous vein. Blood was drawn from the jugular vein for blood counts and smears. At least 2 dogs were started with each vesicant, and if they did not die as a result of the single injection, they were given further injections of the same or increased amounts of the vesicant, or the experiment was discontinued. As a result of the further injections there were fourteen different conditions of dosage to which these dogs were subjected. The amounts and the times of dosage are given in the tables. The vesicants were injected in about 0.4 cc. of solvent.

TABLE 1.—*Blood: Effects of Intravenously Injected Bis(2-Chloroethyl) Sulfide (Code Name, H)* *

A. White Blood Corpuscles

		White Blood Corpuscles †		Neutrophilic Granulocytes †						
	Day	Counts	Mean	Counts	Mean	Percentage Immature	Lymphocytes †	Eosinophilic Granulocytes †	Basophilic Granulocytes †	Metarubricytes ‡
Prior to injection (control)	..	6	100 ± 2.4	6	100 ± 2.6	15.0 ± 2.5	100 ± 7.1	100 ± 6.4	0:600	0:600
After injection.....	1	2	65 ± 4.0§	6	63 ± 1.9§	13.0 ± 2.4	54 ± 6.0§	100 ± 35.0	16:600	0:600
	2	2	63 ± 6.0§	6	69 ± 4.1§	8.0 ± 1.2§	20 ± 5.4§	117 ± 23.0	5:600	0:600
	3	2	54 ± 7.0§	6	62 ± 5.7§	11.0 ± 1.1	26 ± 8.0§	40 ± 6.4§	7:600	0:600
	4	2	47 ± 0.0§	6	52 ± 4.0§	9.5 ± 0.8§	29 ± 10.0§	34 ± 19.0§	2:600	0:600
	5	2	45 ± 2.0§	6	46 ± 1.9§	8.8 ± 2.2§	30 ± 9.0§	35 ± 9.2§	1:600	0:600
	7	2	23 ± 7.0§	6	17 ± 2.8§	21.0 ± 2.5	30 ± 7.0§	27 ± 11.0§	0:600	14:600

B. Red Blood Corpuscles and Thrombocytes

	Day	Counts	Red Blood Corpuscles †	Thrombocytes †
Prior to injection (control).....	..	6	100 ± 0.7	100 ± 1.1
After injection.....	1-5	10	94 ± 2.3§	97 ± 4.1
	7	2	81 ± 4.0§	52 ± 15.0§

* Dogs 11 and 12 were used. The dose was 0.3 mg. per kilogram of body weight.
† The average cell count per cubic millimeter of circulating blood is given as the percentage of the control mean on days following the injection. Each average is followed by its standard error.
‡ The incidence per number of white blood cells counted in smears is given.
§ The number is significantly less than the control mean.

Blood counts and smears were made daily when possible during the first week after injection and on several days of the following weeks. On the death of the animal, the mesenteric and peripheral lymph nodes, the spleen, the adrenal glands and the thymus were removed in all cases; in most cases the tonsils were taken; and in some cases pieces of liver, ileum, cecum and colon, and in 1 case pieces of pancreas and kidney, were taken. The spleen and the adrenal glands were weighed. These organs were fixed in Helly's fluid, and sample sections were stained with hematoxylin and eosin, Feulgen's stain or Mallory's stain. The sternum and the ribs were split, and touch smears of the marrow were made and stained with the May-Giesma method; other parts of the bone and marrow were fixed in Helly's fluid, decalcified, sectioned and stained as were the other organs. The organs of 4 young dogs were used as controls. The methods by which quantitative studies were made of the organs are given in their respective sections and in the tables. The standard errors of the means of the values

TABLE 2.—*Blood: Effects of Intravenously Injected Ethyl-Bis(2-Chloroethyl) Amine (Code Name, HN-1)**

A. White Blood Corpuscles

	Day	White Blood Corpuscles † Counts	White Blood Corpuscles † Mean	Neutrophilic Granulocytes † Counts	Neutrophilic Granulocytes † Mean	Neutrophilic Granulocytes † Percentage Immature	Lymphocytes †	Eosinophilic Granulocytes †	Basophilic Granulocytes ‡	Metarubricytes ‡
Prior to injection (control)	..	9	100 ± 15.0	9	100 ± 18.0	22.0 ± 2.3	100.0 ± 15.0	100.0 ± 31.0	0:900	0:900
After injection.....	2	2	59 ± 17.0§	6	64 ± 9.2§	9.5 ± 2.3§	4.7 ± 1.7§	35.0 ± 14.0§	0:600	0:600
	3	2	47 ± 9.0§	6	53 ± 5.0§	7.9 ± 0.9§	14.0 ± 6.1§	60.0 ± 19.0	0:600	0:600
	4	2	40 ± 15.0§	6	47 ± 6.0§	20.0 ± 3.5	6.7 ± 2.7§	6.0 ± 3.7§	0:600	1:600
	5	2	18 ± 10.0§	6	20 ± 6.0§	35.0 ± 9.5	9.0 ± 1.2§	4.0 ± 2.4§	0:600	0:600
	6-7	2	16 ± 9.0§	6	13 ± 0.8§	34.0 ± 5.7	35.0 ± 3.1§	3.2 ± 0.9§	0:600	0:600
	11-18	2	61 ± 6.0§	6	64 ± 3.0§	25.0 ± 2.2	40.0 ± 12.0§	46.0 ± 12.0§	0:600	0:600
	25-31	2	43 ± 8.0§	6	46 ± 3.0§	17.0 ± 2.4	30.0 ± 6.7§	31.0 ± 13.0§	0:600	0:600
	39-46	2	95 ± 5.0	6	100 ± 2.5	24.0 ± 1.7	70.0 ± 8.3	61.0 ± 27.0	0:600	6:600

B. Red Blood Corpuscles and Thrombocytes

	Day	Counts	Red Blood Corpuscles †	Thrombocytes †
Prior to injection (control).....	..	10	100 ± 2.6	100 ± 4.5
After injection.....	2	2	82 ± 0.0*	112 ± 5.0
	3-4	4	80 ± 2.0*	55 ± 12.0*
	5-7	4	75 ± 2.6*	19 ± 3.0*
	11-46	6	87 ± 4.0*	90 ± 8.9

* Dogs 5 and 6 were used (dog 6 died on the sixth day). The dose was 1.0 mg. per kilogram of body weight.
† The average cell count per cubic millimeter of circulating blood is given as the percentage of the control mean on days following the injection. Each average is followed by its standard error.
‡ The incidence per number of white blood cells counted in smears is given.
§ The number is significantly less than the control mean.

TABLE 3.—*Blood: Effects of Intravenously Injected Tris(2-Chloroethyl) Amine (Code Name, HN-3)**

A. White Blood Corpuscles

	Day	White Blood Corpuscles † Counts	White Blood Corpuscles † Mean	Neutrophilic Granulocytes † Counts	Neutrophilic Granulocytes † Mean	Neutrophilic Granulocytes † Percentage Immature	Lymphocytes †	Eosinophilic Granulocytes †	Basophilic Granulocytes ‡	Metarubricytes ‡
Prior to injection (control)	..	16	100 ± 2.1	16	100 ± 2.7	18 ± 3.1	100 ± 5.0	100 ± 6.6	0:1600	0:1600
After injection.....	1-3	15	59 ± 3.6§	36	89 ± 4.5	14 ± 1.4	10 ± 0.9§	45 ± 6.8§	2:3600	0:3600
	4-7	12	15 ± 1.8§	36	19 ± 1.6§	31 ± 3.7#	9 ± 0.8§	14 ± 1.7§	1:3600	0:3600
	8-9	5	38 ± 11.0§	8	25 ± 13.0§	60 ± 7.8#	16 ± 4.0§	15 ± 3.6§	0:800	20:800
	10-14	10	60 ± 5.0§	16	94 ± 6.8	36 ± 2.7#	21 ± 4.1§	35 ± 7.0§	0:1600	2:1600
	15-45	14	73 ± 5.2§	26	100 ± 6.3	26 ± 1.8	27 ± 3.3§	72 ± 8.4	4:2600	7:2600

B. Red Blood Corpuscles and Thrombocytes

	Day	Counts	Red Blood Corpuscles †	Thrombocytes †
Prior to injection (control).....	..	16	100 ± 0.7	100 ± 2.7
After injection.....	1-3	16	100 ± 0.9	108 ± 2.7
	4-5	8	92 ± 2.0*	96 ± 3.1
	7-14	20	81 ± 2.1*	55 ± 5.4*
	15-45	14	80 ± 2.3*	91 ± 5.0

* Dogs 1 and 2 were used through the forty-fifth day; dogs 13 and 14, through the fourteenth day. The dose was 1.0 mg. per kilogram of body weight.
† The average cell count per cubic millimeter of circulating blood is given as the percentage of the control mean on days following the injection. Each average is followed by its standarderror.
‡ The incidence per number of white blood cells counted in smears is given.
§ The number is significantly less than the control mean.
# The number is significantly greater than the mean of the control.

measured were calculated wherever possible and appear in the tables following the means. At the suggestion of the editor an attempt has been made to make the nomenclature of the blood cells conform with the revisions made by the Committee for Clarification of Cells and Diseases of the Blood and Blood-Forming Organs.[3a,b]

EXPERIMENTAL RESULTS

White Blood Corpuscles of the Peripheral Blood.—The relative changes in the distributions of the total number of white blood corpuscles and of the neutrophilic granulocytes, lymphocytes and eosinophilic granulocytes per cubic millimeter of peripheral blood in the dogs poisoned with the 2-chloroethyl vesicants are presented in tables 1 to 3. The averaged counts are listed as percentages of the means of counts made several days before injection (control means). Each count of each dog was calculated in percentage of the average control count of that dog. These percentages were then averaged with the percentages of the other dogs in the group and the results listed as the mean percentages with standard errors as given in the tables. When only one count was available, the standard error entered was that for the control count. The total counts of the white blood cells were made with standard blood-counting apparatus. The total counts of granulocytes and lymphocytes were calculated from the percentage distributions of these cells in the smears and from the total white blood cell counts. At least three differential counts of 100 cells per count were made for each mean listed in the tables. The immature neutrophils are accounted for as mean percentages of their incidences in the neutrophilic populations of the smears. The method of presenting the data in percentages of the respective means of the controls was used for the purpose of contrasting the relative changes between the several groups of poisoned dogs. Because of the low incidence of monocytes and of basophilic granulocytes in the normal blood, no records of the distributions of the monocytes are entered in the tables, but the incidences of the basophils have been entered to show trends, although their distributions are not statistically significant.

In the following discussion the data will be presented with the idea of showing, if possible, the respective roles of the several types of cells in the total picture. Also, for brevity, the qualification of milligrams per kilogram of body weight will be understood to follow the numerical reference to the amount of vesicant intravenously injected. From analysis of the data presented in tables 1 to 3 the differential effects of the vesicants on the counts of the white blood corpuscles of dogs which survived for at least a week after the initial injection have been contrasted. H (0.3) (table 1) caused leukopenia immediately as a result of moderate neutropenia and lymphopenia. The neutropenia gradually became more severe and was greater at the end of the week than at the beginning, while the lymphopenia did not change. Eosinophilic granulocytes decreased slowly, and there was no marked shift to the left of the immature neutrophils until the seventh day. Marked basophilia occurred during the first three days. Reinjection of H (0.3) on the seventh day after the initial injections aggravated these conditions and was fatal.

HN-1 (1.0) caused leukopenia immediately as a result of moderate neutropenia and extremely severe lymphopenia (table 2). The neutropenia gradually increased in severity, and there was a marked shift to the left of the immature neutrophils on the fourth day, which continued through the seventh day. There was alleviation of the lymphopenia on the seventh day. Eosinophils decreased more rapidly and reached a lower level than in the H (0.3) poisoned dogs. There was no basophilia.

3b. J. A. M. A. **139**:175 (Jan. 15) 1949.

The effects of HN-3 (1.0) (table 3) on the white blood cells are differentiated from those in both the H (0.3) and HN-1 (1.0) poisoned dogs by the moderate initial leukopenia produced only by severe lymphopenia; by the severe acute neutropenia on the fourth day, and by the marked shift to the left of the immature neutrophils on the fourth day. The effects of HN-3 (1.0) agree with those of HN-1 (1.0) and not with those of H (0.3) in producing a rapid decrease in eosinophils and in not affecting the basophils.

From these results it would appear that H (0.3) and HN-1 (1.0) have a moderate immediate inhibitory effect on the bone marrow's production of neutrophils. HN-3 (1.0) has a slower but more harmful effect. HN-1 (1.0) and HN-3 (1.0) have a greater inhibitory effect on the production of lymphocytes than does H (0.3).

In dogs poisoned with H (0.5) the initial neutropenia was relatively greater than after H (0.3), but the lymphopenia was of about the same degree. The eosinophils were also more depressed by the increase in the dose. Basophilia was not so marked as after H (0.3). In the dogs poisoned with H (1.0) there were initially severe lymphopenia and significant decreases in eosinophils and immature neutrophils. Basophilia was present. Significant neutropenia did not occur until the third day and was acute. All the cells decreased on this day. On the fourth day there were practically no leukocytes in the blood. These conditions indicate that increase in the amount of H injected has a greater initial effect on the lymphocytes than on the granulocytes, but neutropenia occurs acutely and is much more severe than that caused by smaller amounts of the vesicant. The terminal conditions are more drastic than those following repetition of H (0.3).

After the first week, beyond which only the HN-1 (1.0) and HN-3 (1.0) poisoned dogs were followed without further injections, the remission of the neutropenia began sooner in the HN-3 (1.0) dogs than in the dog poisoned with HN-1 (1.0), and the neutrophils reached control level by the tenth day in contrast with the thirty-ninth day in the HN-1 (1.0) poisoned dog. On the other hand, a slight remission of the lymphopenia occurred in the HN-3 (1.0) dogs, compared with the practical alleviation of the lymphopenia in the HN-1 (1.0) dog. In both groups the eosinophils fluctuated throughout the remainder of the period, and although they increased, their distributions were much more variable than those of the other cells. The shift to the left of the immature neutrophils continued for a longer period in the HN-3 (1.0) dogs than in the HN-1 (1.0) dog.

No further experiments were carried on with HN-1, but dogs given an initial injection of HN-3 (1.0) were given further injections of the same amount or increasing amounts of HN-3. No detailed description of the conditions incident to these injections will be given here, but so long as the dogs survived the further injections all had the same pattern of severe initial lymphopenia, and most showed delayed but severe neutropenia on the third and fourth days, followed by more rapid remission of the neutropenia than of the lymphopenia. Also, a shift to the left of the immature neutrophils occurred before remission of the neutropenia. In 1 dog which was repeatedly given HN-3, there was continuous basophilia through the course of several injections, but it gradually faded out. Increasing the amount of HN-3 to 1.2 and 1.5 mg. per kilogram and giving it in single injections produced lethal effects, but the initial changes in the white blood cells were the same as those following smaller doses. Quantitative study of the smears showed that there was no evidence of increase of degenerated white blood cells in the blood of the poisoned dogs. There were no changes in the incidences of the monocytes of the peripheral blood during the experimental period.

Red Blood Corpuscles of the Circulating Blood.—The red blood corpuscles of the circulating blood were counted by standard methods, and the counts are presented as percentages of control counts as were those of the white blood corpuscles. Part B of tables 1 to 3 shows that mild anemia followed poisoning of the dogs. HN-1 (1.0) immediately caused moderate anemia; H (0.3), mild anemia; but HN-3 (1.0) had no immediate effect. Subsequently, the anemia in the HN-1 (1.0) poisoned dogs remained at the same level, but in the H and HN-3 poisoned dogs the red blood corpuscles decreased in number until the degree of anemia was equal to that in the HN-1 poisoned dogs. During the periods when the red blood corpuscles of these dogs were studied, there was no remission of the anemia, and it became slightly more severe if the dogs were given further injections of the same or larger amounts of HN-3. The conclusion is drawn that a certain number of erythropoietic cells are injured initially, and although the bone marrow may regenerate, there is never sufficient recovery of the production to replace the lost cells. The crises in marrow regeneration are indicated by the showers of metarubricytes in the peripheral blood which occurred on the seventh day in the H (0.3) poisoned dogs (table 1, A), from the ninth to the fifteenth day in the HN-3 (1.0) group (table 2, A) and not until the sixth week in the HN-1 (1.0) poisoned dog (table 3, B). Quantitative study of the sizes of the red blood corpuscles in the smears showed that there were no differences between the incidences of variations in size of these before and after poisoning.

Thrombocytes of the Circulating Blood.—The thrombocytes were counted in the usual manner and counts were made at the same time that the red blood corpuscles were counted. All the vesicants produced thrombopenia. The distributions of the thrombocytes are listed in part B of tables 1 to 3. In contrasting the effects of the several agents it will be seen that HN-1 (1.0) (table 2, B) produced thrombopenia sooner after injection than did H (0.3) (table 1, B) or HN-3 (1.0) (table 3, B). About the same percentage decrease occurred in all groups, but in those poisoned with HN-1 (1.0) remission was more rapid than in those poisoned with HN-3 (1.0). Further injection of H (0.3) was done before there was remission and resulted in an increase of the severity of the thrombopenia at once, while further injection of HN-3 following remission resulted in the same pattern of thrombopenia and remission as the initial injection. In the dogs poisoned with HN-3 (1.2) and HN-3 (1.5) the thrombopenia occurred at about the same time as in those poisoned with smaller doses, but the dogs did not live long enough to show remission.

Lymph Nodes.—Since the lymph nodes of all the dogs poisoned with vesicants except one were removed at autopsy at least three days after the first injection of the vesicant, the histologic changes which were observed are believed to be secondary to the initial changes produced by the vesicants. In the single dog which died one day after poisoning with HN-3 (1.5), the mediastinal and mesenteric nodes, appearing grossly as large, dark reddish masses, showed degenerative changes which were markedly different from those observed in nodes of rats at the end of the first day after injection of HN-3 (1.0). In both dog and rat the lymphocytes of the nodules and general parenchyma showed much karyorrhexis; many degenerated lymphocytes had been ingested by macrophages of reticulum cell origin. In the rat,[1] the nodules at this initial stage showed viable macrophages, and the nodules were full of dying lymphocytes in all stages of karyorrhexis; but in the dog, the nodules were identified only by degenerated remnants of macrophages, and practically no lymphocytes were present. The conditions of cell necrosis and macrophage activity in the parenchyma outside

of the nodules were the same in both rat and dog, but in the dog the general destruction seemed to have been greater than in the rat. In the other degenerative characteristics there was a marked difference between the nodes of the two animals. In the dog, but not in the rat, the peripheral, intermediate and medullary sinuses were congested, and many of them were full of fibrin. As a result of the congestion of the sinuses, the medullary cords were narrowed. In both dog and rat undamaged plasmacytes were present in the medullary cords. In the dog scattered hemosiderin masses in the medullary cords indicated that there had been hemorrhage in this region. Neutrophilic granulocytes were scattered through the congested sinuses of the nodes of the dog.

After these initial changes there were several directions in which the lymph nodes of the poisoned dogs had changed. The most common type of

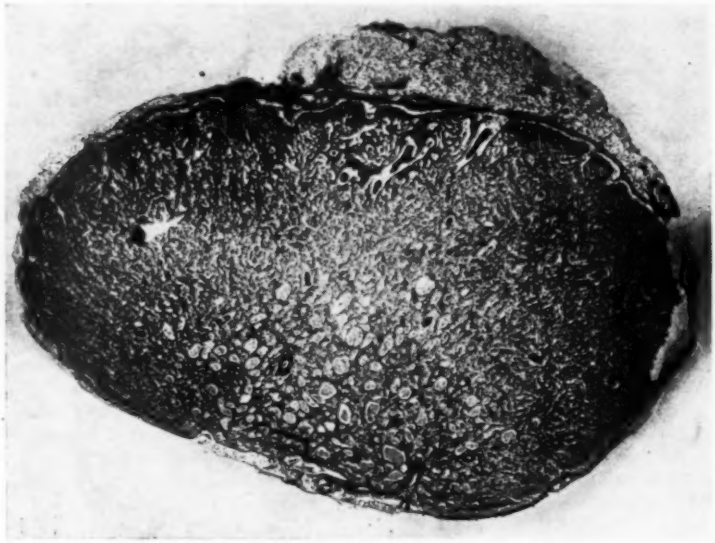

Fig. 1.—Photomicrograph of a median longitudinal section of a mesenteric lymph node from dog 1, which died three days after a second injection of 1.0 mg. of tris(2-chloroethyl) amine per kilogram of body weight given forty-five days after the first injection of the same amount of vesicant. Mallory's stain; section, 7 microns thick; × 13.

degeneration observed was that in which there was extensive hemorrhage, apparently following the initial congestion. As a result of the hemorrhage the parenchyma was replaced by red blood corpuscles and fibrin. Mediastinal and mesenteric lymph nodes from all 6 dogs poisoned with H, and the nodes from 6 of 9 dogs poisoned with HN-3, showed these hemorrhagic changes. The hemorrhages obliterated the cortex, compressed the medullary cords and filled the sinuses with red blood corpuscles. In these nodes there was no trace of nodules. Remnants of medullary cords containing plasmacytes were present. No granulocytes were observed in any of the nodes. In the nodes

of one of the H poisoned dogs there were areas of bacteria surrounded by necrotic tissue. In most of the nodes, the capsule and the trabeculae were markedly thickened, and there was some fibrosis around the hilus.

The second most characteristic change in the lymph nodes was one in which practically all of the lymphocytes were absent and only the collagenous stroma persisted (fig. 1). The nodes looked as though the lymphocytes had been washed out. The reticulum stroma of the whole lymphatic tissue was beautifully preserved, and the framework of the nodules was particularly well delineated. Scattered reticulum cells remained clinging to the fibers or lying free in the reticulum-lined spaces. The courses of the vasa could be readily traced from the hilus. Despite the absence of lymphocytes, the nodes were not collapsed, a condition probably resulting from the presence of some fibrin in the reticulum spaces. These conditions were the major features in certain of the nodes in 3 of the 6 dogs given injections of H, in the nodes from the dog given HN-1 and in the nodes of 3 of the 9 dogs poisoned with HN-3.

The third type of degeneration might be called a slightly modified continuation of the initial changes. In these nodes there were remants of the nodules; the parenchyma was loose, but contained small lymphocytes; the medullary cords were well preserved and contained many plasmacytes and there was more or less fibrotic invasion of the cortex and the hilus. The trabeculae and the capsule were usually thicker than normal. Such nodes were present in 3 dogs poisoned with HN-3.

A fourth type of node, characterized by areas of regeneration of the lymphocytes of the cortex, was found in only 1 dog, and it was the one which lived for one hundred and fifty-two days after the initial injection of HN-3 (1.0). This dog survived four injections and succumbed eighteen days after the fifth injection. Grossly, these nodes were small and white, and there was no fat around them. In the submaxillary and mesenteric lymph nodes of this dog, the capsule and the trabeculae were thicker than normal, and the sinuses were wide and open. The peripheral margin of the cortex was composed of a dense mass of lymphatic tissue, in which many lymphocytes were in mitosis. The medullary cords were composed for the most part of plasmacytes with few lymphocytes. Among these cells were hemosiderin masses, which are believed to be evidence of hemorrhage in this region. A mediastinal node from this same dog showed a smaller amount of regenerating lymphatic tissue, more connective tissue and catarrh of the sinuses.

The histologic conditions observed in the lymph nodes of these poisoned dogs indicate that the initial change is destruction of lymphocytes, accompanied by vascular congestion. The initial changes may be followed by hemorrhage, which prevents regeneration, or by complete washing out of the lymphocytes, leaving no centers for regeneration, or by partial recovery of the lymphatic tissue and regeneration of the lymphocytes. In the lymph nodes of only 1 dog was there evidence of regeneration of the lymphatic tissue, and this dog survived four injections of HN-3. From these facts it would appear that the continued lymphopenia observed in these dogs was caused by the failure of the lymphatic tissue to regenerate, since increase in the number of lymphocytes in the blood occurred only in that dog which showed regeneration in the lymph nodes. Except for this dog, the lymph nodes of the dogs differed from those of the rats in their reaction to the mustard vesicants. In the rats, the secondary changes, such as congestion and hemorrhage, did not occur, and the lymphatic tissue, after its initial intoxication, gave evidence that it could regenerate; and even though the nodes were small, they could still be drained of sufficient lymphocytes to prevent the persistent lymphopenia that occurred in most of the dogs.[1] Hence it is believed that the secondary changes in the lymph nodes, such as congestion and hemorrhage, are

important factors in limiting the production of lymphocytes and in permitting continuance of the severe lymphopenia observed in these dogs.

In the lymph nodes of both dog and rat it has been found that the plasmacytes of the medullary cords were remarkably resistant to the poisonous effects of the vesicants. Plasmacytes are usually regarded as decending from lymphocytes and as representing an involutional stage of these cells. In the control dogs the plasmacytes were never so mitotically active as were the lymphocytes. Such conditions suggest that their resistance to the vesicants is caused by the stable condition of their chromatin, as it is known that vesicants and roentgen rays have a selective effect on cells in which the chromatin is in an active condition and do not injure the cells of more stable tissues, such as connective tissue cells, reticulum cells, etc., directly.

Spleen.—The spleen of the dogs poisoned with the vesicants showed when examined grossly evidences of fibrosis of the capsule. The shape of the spleen was modified in only 2 dogs; in one dog it had a large hematoma, and in the other it was deformed from becoming entangled with, and adherent to, the mesentery. Except in 2 dogs poisoned with H (0.3), in which the relative weights of the spleens were considerably greater than normal, the range of the relative weights of the spleens (from 1.33 to 2.42 Gm. per kilogram of body weight) was not significantly different from the range of weights given for 14 thymectomized and 14 control dogs aged from 2 to 16 months (1.03 to 2.64 Gm. per kilograms of body weight [5]).

Histologic examination of the spleens of the poisoned dogs showed that all were in a condition of passive congestion, characterized by hyaline collagenous thickening of capsule and trabeculae, open and broken venous sinuses, congestion and fibrosis of the pulp spaces and stasis of the blood in the larger veins of the trabeculae. Examination of the arteries showed that in all the spleens there were atheromatous changes in the walls of the arteries which could have caused the congestion. The larger arteries did not show consistent changes, although the muscle cells of the media of the trabecular arteries were usually infiltrated with fat. But in the smaller arteries the endothelial cells were vacuolated and sloughed into the lumens; the media was markedly infiltrated with fat, and in the ellipsoids the capsule was usually obliterated by hemorrhage. Hence it is believed that the passive congestion present in these spleens was caused by pathologic changes of arteries and capillaries.

Detailed examination of the pulp showed that together with the congestion and fibrous infiltration of the pulp spaces there occurred scattered hemosiderosis, absence of lymphocytes and granulocytes, scattered persistence of reticulum cells, many of which had pyknotic nuclei, and presence of macrophages in variable stages of degeneration with and without hemosiderin. The degenerated cells were not accumulated into masses. Monocytes and plasmacytes were present in some spleens but were never numerous or massed. In several spleens there were areas of necrotic degeneration within which rod-shaped bacteria were observed. These areas infested with bacteria indicate that when pathologic changes occur incident to poisoning with the vesicants, bacterial activity, which might be held in check by the activity of surrounding tissues receiving a normal blood supply, may increase in virulence when this supply is cut off by the degenerative changes produced in the vasa by the vesicants. The presence of these bacteria also implies that in vivo the vesicants are not bactericidal.

In addition to the congestive degeneration, the spleen showed marked evidence of degenerative changes in the lymphoid tissue. The malpighian corpuscles of the spleens of all dogs showed definite evidence of degeneration. This degeneration

in the lymphoid nodules was characterized by small size, looseness, lack of medium-sized lymphocytes, pyknosis of the nuclei of small lymphocytes and of many of the reticulum cells, fragmentation of the cytoplasm of the reticulum cells and macrophages, much edema, hemorrhage within the center and around the margins of the nodules and absence of mitosis in the cells present. The congestion could have caused the hemorrhage and the reduction in the size of the nodules, but the direct action of the toxic vesicants is presumed to have damaged the lymphocytes before the secondary effects occurred. The nodules appeared to be in such a degenerated condition that there seemed little chance that they would recover activity and resume production of lymphocytes.

The lymphoid cords surrounding the arteries of the pulp were markedly reduced in amount in the spleens of all the dogs. The reticulum cell stroma remained, and within its meshes there were a few lymphocytes, usually with pyknotic nuclei, and some plasmacytes. The cords were usually separated from the surrounding pulp by flattened reticulum cells and fibers. The tissue was free of hemorrhage, but in some places it contained masses of hemosiderin or macrophages with hemosiderin.

The volume of the lymphoid tissue, including the malpighian corpuscles, which in sample sections of the spleens of the normal dogs amounted to about 4.4 per cent of the total volume, was lower in the spleens of all the poisoned dogs. The volume of this tissue was least in the dogs given injections of H (range, 0.7 to 3.0 per cent) and in the dogs poisoned with the greatest amounts of HN-3 (range, 0.8 to 1.1 per cent). Taking into consideration these data and those from the qualitative study, one may conclude that the vesicants not only damaged the cells but reduced the amount of lymphoid tissue.

The pathologic changes in the spleens of these dogs poisoned with vesicants were much greater than those which occurred in the rats poisoned with the same vesicants in about the same dosage.[1]

Tonsils.—None of the histologic features characteristic of the normal tonsil were seen in sections of the tonsils of the dogs poisoned with vesicants. Unfortunately, the tonsils were not taken from all the dogs, but sufficient material was obtained to give an idea of the changes which followed the injection of the vesicants. Initially, as in the lymph nodes, the poisoning was followed by destruction and phagocytosis of the lymphocytes, obliteration of the topography of the nodules and congestion of the vasa. This is a picture of cell destruction and inflammation. Such a condition is usually followed by involution, characterized by further reduction in the amount and the regular arrangement of the lymphoid nodules, dissolution of the dead phagocytes, thinning of the epithelium, increase of the amount of connective tissue and development of plasmacytes. Plasmacytes were occasionally seen in the normal tonsil, but they never formed layers and sheets such as were present beneath the epithelium of the tonsils of the poisoned dogs. These conditions usually occurred by the third day after poisoning. If the dog survived for a longer period, the tonsils shrank, the connective tissue increased, the epithelium became thicker, and the remnants of the lymphoid tissue began to undergo regeneration. The masses of regenerating lymphocytes were composed of mingled reticulum cells and lymphocytes. These masses lay near the longitudinal axes of the tonsils. Peripheral to the masses were regions characterized by large numbers of plasmacytes, which permeated the fibrotic stroma. It appeared that as the lymphocytes were produced they became modified into plasmacytes, since there was no evidence of local proliferation of the plasmacytes.

The lymphocytes of the tonsils were initially poisoned by the vesicants, as were those of the lymph nodes and the spleen, but the subsequent changes in the tonsils differed from those in both lymph nodes and spleen in the absence of hemorrhage. The tonsils were further different from the lymph nodes in retention of lymphocytes and in a tendency to produce large numbers of plasmacytes in the region formerly occupied by the nodules, whereas in the lymph nodes the plasmacytes, while persisting, were confined to the medullary cords.

Thymus.—In the normal adult dog the thymus is in a condition of involution [4] and does not contribute to the lymphocyte population of the blood.[5] In the dogs given injections of vesicants the thymus when present was in a condition of involution characterized by shrunken lobules composed of a loose reticulum stroma containing few lymphocytes, hydropic macrophages and congested vasa. The lobules were separated by edematous connective tissue. In some of the dogs there was no trace of the thymus. It is possible that the vesicants had speeded up the process of involution as they do in the rat, but the changes in the structure of the thymus apparently had nothing to do with the lymphopenia of the peripheral circulation.

Bone Marrow.—The marrows of the ribs and the sternum of 4 young dogs served as controls for conditions in the bone marrows of the poisoned dogs. Stained sections of marrow were used for histologic study and for making counts of cells per 500,000 cubic microns. The percentage distributions of the different types of cells were determined from counts of 500 cells from each region of each dog in touch smears, dried and stained by the May-Giemsa method. In a few of the poisoned dogs the marrows of femur and vertebrae were studied. The numbers of different types of cells per unit volume (500,000 cubic microns) were calculated from these data and are presented in table 4. Myeloid and erythroid cells in mitosis per 25 unit volumes (50,000 cubic microns per unit volume) were counted from each region of the sectioned material.

In the sternal marrow of the control dogs, the cells were closely packed, and there were few open sinuses and little fat (fig. 2). The average number of cells per unit volume in the sternal and rib marrow was 1,960 $\pm$ 82 (table 4). The myeloid-erythroid ratio in the sternum was 0.9:1.0; that in the ribs, 1.5:1.0. The sternal marrow was less myeloid than that of the dogs studied by Alexandrov [6]; the ratio in the rib marrow agreed with that of Mulligan [7] on young dogs, but was less myeloid than the marrow of the dogs studied by Stasney and Higgins.[8] Since the cells in the marrow of the poisoned dogs were so few, the separate classes of myeloid and erythroid cells have not been entered as such in table 4, but all of the immature myeloid cells and erythroid cells have been classified as myeloid and erythroid cells, respectively. Cells which had little or no representation in the normal marrow but were present in the marrow of the poisoned dogs included degenerated myeloid cells, macrophages with hemosiderin debris, and plasmacytes.

In the bone marrow of all the poisoned dogs at death there was cellular hypoplasia (fig. 2, *B*). There was considerably more fat in the marrow of these dogs than in that of the controls. In most of the dogs the marrow was hyperemic, and the surviving cells were scattered between the fat and the congested sinusoids.

4. Park, E. A., and McClure, R. D.: Am. J. Dis. Child. **18**:317, 1919.
5. Hammar, A.: Endocrinology **5**:543, 1921.
6. Alexandrov, A. F.: Folia haemat. **41**:428, 1930.
7. Mulligan, R. M.: Anat. Rec. **79**:101, 1941.
8. Stasney, J., and Higgins, G. M.: Am. J. M. Sc. **193**:462, 1937.

Fibrin deposits in places formerly occupied by cells were common. There were no islets of erythroid cells even in the marrow of those dogs in which these cells survived in greater numbers than in others in which they were few. The numbers of megakaryocytes were greatly reduced.

Qualitatively the myeloid cells which were present showed many signs of degeneration, such as vacuolated nuclei and cytoplasm, coarseness of chromatin, swelling of the nuclei and deficiency of granules in the cytoplasm. In some dogs, particularly those poisoned with HN-3, there were large numbers of myeloid cells which were counted as degenerated and in which there seemed to be cessation of growth and differentiation of the cytoplasm. Macrophages with hemosiderin and other debris were more abundant in the poisoned dogs than in the controls (table 4). In the myeloid cells mitosis wes suppressed, and in most of the marrows there was no evidence of regeneration. There was practically no mitosis in the erythroid cells. The incidences of erythroid cells were reduced practically to zero in the H poisoned dogs, but showed greater degrees of survival in those

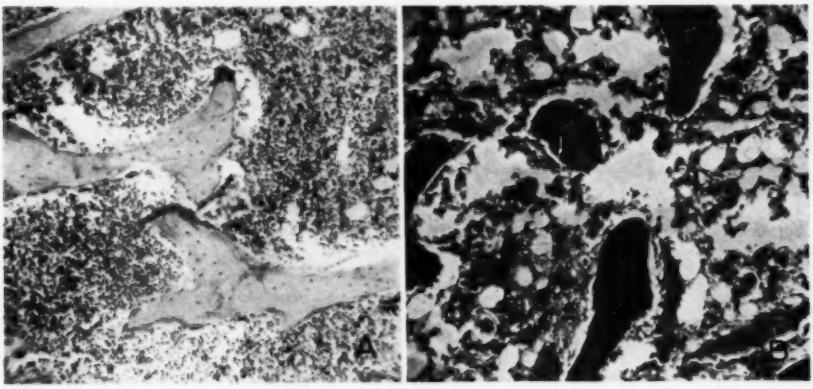

Fig. 2.—Photomicrographs of sections of marrow of the sternum. *A*, marrow from a control dog; hematoxylin and eosin; section, 5 microns thick; × 90. *B*, marrow from dog 7, which died four days after injection of 1.0 mg. of bis(2-chloroethyl) sulfide per kilogram of body weight; Mallory's stain; section, 5 microns thick; × 90.

poisoned with HN-1 or HN-3 (table 4). The greatest survival of erythroid cells occurred in the HN-3 poisoned dogs. Myelocytes were not as uniformly reduced in number as were the erythroid cells, and the greatest relative survival of myelocytes was in the dog poisoned with HN-1 (table 4).

A quantitative study of the sizes of the myelocytes in the poisoned dogs in contrast with those in the controls showed that there was no significant swelling of the myelocytes in the H poisoned dogs but that there was in the dog poisoned with HN-1 and in one of the dogs poisoned with HN-3.

First, if it is assumed that in the dog the life tenure of the neutrophilic granulocytes is about four days, as it has been estimated to be in the rabbit,[9] then if the dogs survived poisoning for four days and there was a condition of extreme

9. Weiskotten, H. G.: Am. J. Path. **6**:183, 1920.

TABLE 4.—*Bone Marrow: Effects of Intravenously Injected Bis(2-Chloroethyl) Sulfide (H), Ethyl-Bis(2-Chloroethyl) Amine (HN-1) and Tris (2-Chloroethyl) Amine (HN-3)**

Dog	Vesicant	Dose, Mg. per Kg. of Body Wt.	Injections	Days Elapsing †	Total Number of Cells	Myelocytes	Neutrophilic Myelocytes	Eosinophilic Myelocytes	Degenerated Myelocytes	Metarubricytes Rubricytes	Reticulum Cells	Macrophages	Lymphocytes	Plasmacytes
Control	.....	...	..	..	1,960 ± 82	700 ± 65	138 ± 18	86 ± 11	0	790 ± 54	39 ± 8	0	91 ± 17	9
11	H	0.3	1	7	132 ± 29	1 ± 1	1 ± 0	0	0	1 ± 1	11 ± 0	7 ± 2#	48 ± 11	57 ± 15#
		0.3	2	5										
12	H	0.3	1	7	450 ± 100	121 ± 21	3 ± 1	1 ± 1	1 ± 1‖	42 ± 9	72 ± 54‖	14 ± 2#	62 ± 1	101 ± 9#
		0.3	2	5										
9	H	0.5	1	5	172 ± 9	3 ± 1	2 ± 0	1 ± 1	1 ± 0‖	3 ± 1	79 ± 6#	24 ± 0#	23 ± 4	33 ± 3#
10	H	0.5	1	4	130 ± 49	4 ± 1	2 ± 0	1 ± 1	2 ± 1‖	8 ± 6	55 ± 22‖	20 ± 7#	23 ± 6	18 ± 3#
7	H	1.0	1	4	110 ± 26	8 ± 4	3 ± 0	1 ± 1	1 ± 1‖	2 ± 1	53 ± 7‖	10 ± 2#	7 ± 0	21 ± 5#
8¶	H	1.0	1	4	90 ± 43	11 ± 2	2 ± 1	1 ± 0	1 ± 1‖	9 ± 6	21 ± 6‖	5 ± 3‖	11 ± 6	25 ± 24‖
6	HN-1	1.0	1	6	410 ± 20	240 ± 0	55 ± 5	5 ± 4	30 ± 3#	30 ± 0	26 ± 17‖	6 ± 1#	1 ± 1	5 ± 0#
1	HN-3	1.0	1	45	175 ± 40	17 ± 16	2 ± 2	6 ± 6	43 ± 7#	29 ± 28	13 ± 0	9 ± 8‖	7 ± 5	44 ± 33‖
		1.0	2	3										
14	HN-3	1.0	1	14	107 ± 27	1 ± 1	1 ± 0	1 ± 0	0‖	38 ± 4	4 ± 2	4 ± 1#	36 ± 5	0‖
		1.1	2	27										
		1.1	3	3										
2	HN-3	1.0	1	45	325 ± 50	172 ± 50	19 ± 6	1 ± 1	50 ± 33‖	57 ± 3	14 ± 1	1 ± 0‖	2 ± 0	0
		1.0	2	40										
		1.0	3	20										
		1.1	4	28										
		1.1	5	18										
4‡	HN-3	1.2	1	9	288 ± 56	71 ± 5	8 ± 5	2 ± 0	6 ± 2.4	158 ± 47	15 ± 1	3 ± 2‖	2 ± 1	3 ± 1#
15	HN-3	1.5	1	4	91 ± 4	1 ± 1	1 ± 0	0	0	0	48 ± 1	3 ± 1	7 ± 1	31 ± 5#
18	HN-3	1.5	1	4	192 ± 8	3 ± 0	1 ± 1	3 ± 1	40 ± 22	3 ± 2	93 ± 17#	11 ± 1#	8 ± 2	27 ± 10#

* The table shows the distribution of the cells per 500,000 cubic microns of marrow of sternum and ribs (unless otherwise indicated) of the poisoned dogs as contrasted with conditions in 4 normal dogs. The total numbers of cells were counted in sections, and the differential counts were made from smears in which 500 cells were counted from each dog. Each number is accompanied by the standard error of its mean. All numbers except those of the control which are not marked by the symbol # or the symbol ‖ are significantly less than the control mean.

† This column gives the days elapsing between injections or from the time of the first injection to death.

‡ Marrow of a femur was used in addition to that of ribs and the sternum.

¶ Marrow of a femur was used instead of that of the sternum.

‖ The number is not significantly different from the control mean.

The number is significantly larger than the control mean.

neutropenia in the peripheral blood, the marrow may be assumed to have been poisoned to such a degree by the vesicant that it could effect no replacement of neutrophils. Under these conditions there might be myeloid cells in the marrow, but they would remain unproductive. Of 5 dogs poisoned with H, there was only 1 (no. 12, table 4) which would fall into this category; the only dog poisoned with HN-1 (no. 6, table 4) and 2 dogs (nos. 2 and 4, table 4) of 6 poisoned with HN-3 would also be included. Second, if the peripheral blood was agranulocytic and the bone marrow was without myeloid cells at the same time, it may be assumed that, in addition to the normal loss of the granulocytes of the peripheral blood, the myeloid cells of the marrow were destroyed by the poison and that there would be no possibility of regeneration. This condition was present in 4 out of 5 dogs poisoned with H and in 1 of the 6 poisoned with HN-3. Third, if there was a moderate diminution of the granulocytes in the peripheral blood, practically no myelocytes in the bone marrow, and the dogs died before the fourth day after the first injection, or a further injection, it may be assumed that the myelocytes of the bone marrow were destroyed, and that as soon as the normal loss of granulocytes occurred there would be no replacement. This condition was present in 3 of the 6 dogs poisoned with HN-3, and 2 of these were poisoned with the greatest amounts of HN-3 (nos. 15 and 18, table 4). From these analyses it will be seen that H has a greater degenerative effect on the myeloid cells than HN-1 or HN-3 but that HN-3 is more toxic than HN-1.

Using these data for prognosis, one may, it is suggested, make a prognosis unfavorable for survival if the granulocytes of the peripheral blood continue to decrease sharply without replacement after the fourth day of injection. If, however, the neutropenia does not become extreme by the fourth day and begins to be alleviated by the seventh day, the prognosis is favorable. It is also of interest to note that in those dogs in which myelocytes were present in the marrow at death, there was a significant shift to the left of the immature neutrophils just before death or on the day of death. Thus a shift to the left of neutrophils could possibly be prognostic of impending death, provided there was marked neutropenia of the blood at the same time.

There was a consistent relation between the incidences of lymphocytes in the marrow and in the peripheral blood of the poisoned dogs. In the H poisoned dogs, which had higher peripheral lymphocyte counts than the dogs poisoned with other vesicants, there were more lymphocytes in the marrow (table 4). Relative hyperplasia of plasmacytes was consistently present in the H poisoned dogs, occurred to a slight degree in the HN-1 poisoned dog and was of variable occurrence in the HN-3 poisoned dogs (table 4). These plasmacytes appeared to be derived from the lymphocytes and not from reticulum cells.

There seemed to be no consistent relation between the incidence of lymphocytes and the incidence of the myeloid or the erythroid cells in the bone marrow. If it is assumed that normally lymphocytes are drained off into the marrow to serve as ancestors for the myeloid and erythroid cells as hemoblasts,[10] it would be expected that in the marrows of those dogs with the greatest numbers of lymphocytes there would be consistently higher levels of myeloid and erythroid cells, but in the samples studied there is no such relation (table 4).

In all the dogs there was extreme hypoplasia of the erythroid cells in the marrow (table 4). The erythroid cell hypoplasia was relatively much greater than the anemia of the peripheral blood no matter how long the dogs had lived or how many injections they had received. The initial anemia in the peripheral

10. Jordan, H. E.: Anat. Rec. **73**:227, 1939.

blood of the dogs poisoned with H or HN-3 was about what would be expected if the proliferation of the erythroid cells of the whole marrow had been inhibited by the vesicant immediately after injection and there was no replacement of the cells normally lost at the rate of 0.75 per cent of the red blood corpuscles per day with a life tenure for the red blood corpuscles of the dog of about one hundred and twenty-four days.[11] However, in the HN-1 poisoned dogs the anemia was so severe on the second day after injection as to suggest that not only had the poisoning caused inhibition of production but also greater than normal loss of red blood corpuscles of the peripheral blood. The hypoplasia of the erythroid cells in the marrow of the dogs which died at the end of four days after poisoning—e. g., no. 8 (H, 1.0) and nos. 15 and 18 (HN-3, 1.5)—was of such a degree that the destruction of these cells should have shown greater decreases in the numbers of red blood corpuscles in the peripheral blood than was observed. Since no decreases occurred before death, it must be assumed that there are other factors which maintained the numbers of red blood corpuscles despite the poisoning of the erythroid cells of the marrow. The fact that the spleen, which is normally full of red blood corpuscles, was practically ischemic in all of these dogs leads to the inference that the withdrawal of the blood from the spleen would account for the maintenance of the numbers of red blood corpuscles in the peripheral blood. When the dogs survived poisoning for more than four days, the numbers of red blood corpuscles began to decrease, and such decreases could be accounted for by the hypoplasia of the erythroid cells of the bone marrow and their slow regeneration between injections. Finally, as a result of continued poisoning, the regeneration of the erythroid cells was slowed, and although the conditions of anemia imposed by the initial injection did not become more severe, there was no remission. Every time a dog was given another injection, the anemia became aggravated, because there was no longer a reserve of red blood corpuscles in the spleen and more erythroid cells in the marrow were poisoned. Furthermore, judging from the small numbers of erythroid cells present in the marrow of the poisoned dogs at death, one concludes not only that the proliferation was inhibited but that most of the cells were destroyed. HN-1 apparently has a greater immediate destructive effect than H or HN-3, but the extent of terminal destruction may be of the same degree after poisoning with any of the vesicants. The lack of correlation between the numbers of red blood corpuscles in the peripheral blood and the decrease of the numbers of erythroid cells in the marrow resembles closely the conditions found in man treated with therapeutic doses of methyl-bis(2-chloroethyl) amine in whom the quantitative distributions of the cells of the sternal marrow were followed by biopsy.[12]

In all of the dogs in which the marrow was studied there was thrombopenia at death. In those which had only one injection of lethal doses of H, there was moderate to severe thrombopenia, and there were practically no megakaryocytes in the samples of marrow studied. In those which had more than one injection of H, the same condition obtained, and they never recovered from the initial poisoning of the megakaryocytes. In the dogs poisoned with HN-1 there was severe thrombopenia at death and despite the persistence of myeloid and erythroid cells in the marrow, there were no megakaryocytes. Dogs poisoned with the strongest doses of HN-3 in which there was moderate thrombopenia in the blood had some megakaryocytes in the very hypoplastic marrow. In 1 dog which had five

11. Hawkins, W. B., and Whipple, G. H.: Am. J. Physiol. **122**:418, 1938.

12. Spurr, C. L.; Jacobson, L. O.; Smith, T. R., and Barron, E. S. G., in Moulton,[2] p. 24.

injections of HN-3 there was thrombopenia at death although megakaryocytes were still present in the marrow. The persistence of the megakaryocytes probably accounted for the recovery of this dog from the thrombopenia which followed each injection. From these data it would appear that H and HN-1 are more toxic to the megakaryocytes of the marrow than is HN-3 and that the toxic effects are directly related to the thrombopenia of the circulating blood.

Adrenal Gland.—Quantitative and qualitative histologic studies were made of the adrenal glands of the dogs poisoned with vesicants and the conditions contrasted with those observed in the adrenal glands of nonpoisoned dogs. The average relative weight of the glands did not show any significant changes from the average normal relative weight of 0.117 Gm. per kilogram computed from Baker's[13] values.

The adrenal glands of the poisoned dogs all showed evidence of pathologic changes, which appeared to be secondary to damage done to the blood vessels. The changes which have been analyzed quantitatively showed: hyaline thickening of the capsules, decreases in the nuclear and cytoplasmic volumes of the cells of the cortex and decreases in the amount of chromaffin tissue in the medulla. The nuclei of the cells of the medulla were less affected by the poisons than were those of the cells of the cortex. Cell proliferation was practically inhibited, so that there was little possibility of regeneration. Focal necrosis, hemorrhage and congestion were particularly characteristic of the zonae fasciculata and reticularis. Taking all the pathogenic effects into account one concludes that HN-3 had the most intoxicating effect on the cells of the cortex. H and HN-1 had about the same effect. H, however, had the most intoxicating effect on the medulla and HN-1 the least.

Pathologic changes such as have been observed in the adrenal glands of the dogs poisoned with vesicants were not found in the glands of rats poisoned with the same relative amounts of these vesicants.[1] In the rats which survived for four days there was moderate hypertrophy of the cells and enlargement of the vacuoles in the spongy zone of the zona fasciculata. There was some congestion in the zona reticularis, but it was never accompanied by degeneration of cells. Ludewig and Chanutin[14] have shown that in the adrenal glands of such rats the total cholesterol concentration and the total lipid concentration are decreased. Therefore, there seems to be a correlation between the swelling of the cells and the decrease of the substance which may be the basis for the production of the cortical hormone. In the poisoned dogs, the cells in the spongy zone are smaller than in the controls, the vacuoles are enlarged, and there is much focal necrosis and pathologic change in this zone. From a contrast between the histologic aspects of the cortices of the glands of the poisoned rats and dogs the inference is made that the production of the cortical hormone is diminished to a greater degree in the dog than in the rat.

Other investigators who have studied the adrenal glands of guinea pigs intoxicated with sulfur mustard (H),[15] phenol, chloroform, carbon tetrachloride, dichloromethane and tetrachloromethane[16] have reported pathologic changes similar in general to those observed in the adrenal glands of the poisoned dogs. Both Graham[16] and Hoerr[15] have emphasized the fact that the zona reticularis is the site of the earliest and most extensive lesions. Similar pathologic changes

13. Baker, D. D.: Am. J. Anat. **60**:231, 1937.
14. Ludewig, S., and Chanutin, A.: Endocrinology **38**:376, 1946.
15. Hoerr, N.: Am. J. Anat. **48**:139, 1931.
16. Graham, G. S.: J. M. Research **34**:241, 1916.

have been found in the adrenal glands of dogs in which the suprarenal vein was ligated.[17] These dogs showed the same symptoms of general weakness, disturbances of alimentation, etc., as were observed in the dogs poisoned with vesicants.

Since these pathologic changes occurred in the adrenal glands of dogs which had at the same time suffered widespread intoxication of the lymphoid organs, it is believed that the cortical hormone is not responsible for the initial damage of the lymphocytes. These data support the view expressed earlier after a study of the effects of the vesicants on adrenalectomized rats in which intoxication of lymphocytes occurred in the same manner as it did in nonadrenalectomized rats which had been given injections of vesicants.[1]

Other Organs.—No other organs of the poisoned dogs were taken systematically for histologic study, but sample sections were made from some organs which appeared on gross examination to differ from the normal. Among these organs were the small intestine, the cecum, the colon, the liver, the pancreas and the kidney. In 2 dogs which had been poisoned with several injections of HN-3, either the epithelium of the intestinal villi was sloughed or the cells were hypertrophied, as were the cells which had been exposed to roentgen rays.[18] There were no mitoses in the cells of the glands, a condition which would prevent the normal rapid replacement of the epithelial cells of the villi.[19] In the poisoned rats damage to this part of the intestine, it was inferred, offered an avenue for greater loss of blood cells than occurs in the normal animal and would thus contribute to the leukopenia and anemia observed in the peripheral blood.[1] That the same conditions occurred in the dog lends support to this view and may possibly account for the rapid decrease of the lymphocytes of the blood during the first five hours after injection of the vesicant even though the cells are delivered in normal numbers to the blood via the thoracic duct during this period.[20] Not only is it likely that blood is lost through the damaged intestine but it is obvious that material needed in the production of blood cells cannot be normally absorbed from ingested food. The cecum and colon of 2 dogs poisoned with HN-3 showed sloughing and coagulation necrosis of the mucosa. The livers of 2 dogs poisoned with H (0.3) were completely necrotic, and in one of them bacteria were present. In the liver of the dog poisoned with repeated injections of HN-3 there was extensive portal cirrhosis. The pancreas of this dog showed patchy degeneration of the acini and shrinkage of the islands of Langerhans. The right kidney of this dog was only a cystic sac, and the left showed evidences of acute glomerulonephritis with patchy necrosis.

In these dogs, particularly those poisoned with the greatest amounts of the vesicants, there were decrease in the albumin and increase in the alpha globulin fraction of the serum,[3] as well as increases in the plasma fibrin and cholesterol.[4] The latter condition was particularly marked in the dogs given repeated injections of H (0.3), in which there was evidence also of necrosis of the liver.

Observations on the ganglion cells of ganglions of the autonomic nervous system near the adrenal gland indicated that the cells had been damaged. In four ganglions studied there were all degrees of degeneration of cells, primarily of the nucleus and secondarily of the cytoplasm. The nucleolus appeared to be swollen in most of the cells and in several cells it was observed in a position suggesting that it was being extruded through the nuclear membrane. The injured nucleus was wrinkled; the nuclear membrane had blunt projections and was chromatic. The injured

17. Rogoff, J. M.: Am. J. Path. **38**:392, 1944.
18. Warren, S., and Friedman, N. B.: Am. J. Path. **18**:499, 1942.
19. Leblond, C. P., and Stevens, C. E.: Anat. Rec. **100**:357, 1948.
20. Courtice, F. C., and Jones, R. P.: Unpublished data, 1944.

cytoplasm was dense around the nucleus and vacuolated at the periphery, and the cell membrane was indistinct. The capsule and neurilemma cells were chromatic, and the capsules were broken. Such conditions could be a result of congestion and anoxia incident to some process interfering with the blood supply or they could have been caused directly by the vesicant.

These meager observations are presented to suggest that while the vesicants are primarily poisoners of the hemopoietic cells, they also have general toxic effects. From the relations of the pathologic effects it appears that the degeneration observed was secondary to injury of the blood vessels, particularly to that of the capillaries. It is possible that the degeneration observed in the hemopoietic organs is also related to the damage of the capillaries, since changes occur in organs which have extensive capillary nets and the circulating poisons would have had immediate access to the hemopoietic cells if the wall of the capillary was injured.

COMMENT

The descriptive data presented in this paper support the views of various investigators [21] that the beta chloroethyl vesicants exert a selective toxic action on the nuclei of actively proliferating cells of the lymphoid organs and bone marrow in mammals. The results of this action were observed in the quantitative decreases in the cells of the peripheral blood of the poisoned dogs. The extent of cellular intoxication was directly related to the amount of vesicant injected in a manner such as that reported by Karnofsky and associates.[22] With sublethal doses of the vesicants, repeated injection of the dose produced the same pattern of change in the peripheral blood as did the initial injection. The leukopenia of the peripheral blood followed the same pattern as was observed in the victims of the atomic bomb radiation in Japan [23] and in animals exposed to the atomic bomb radiation in test "Able" at Bikini.[24] Also the skeletonized appearance of some of the lymph nodes of the poisoned dogs, consisting largely of reticulum stroma, cell debris, fibrin and macrophages, was like that characterizing the human victims. Similar, too, were the hypoplasia of the bone marrow and the pathologic changes in the spleen. In gross pathologic aspects the lymph nodes and the marrow, but not the spleen, were similar to the same organs of the animals exposed at Bikini.

In contrasting the morphologic aspects of the lymph nodes of rats [1] and dogs which were killed or died at the same time after injection of the same relative amounts of the vesicants, the nodes of the poisoned dogs showed greater pathologic changes and greater loss of lymphocytes than did those of the rats. This greater pathologic effect of the vesi-

21. Gilman, A., and Philips, F. S.: Science **103**:409, 1946. Kindred.[1] Moulton.[2]

22. Karnofsky, D. A.; Burchenal, J. H.; Ormsbee, R. A.; Cornman, I., and Rhoads, C. P., in Moulton,[2] p. 11.

23. Le Roy, G. V.: J. A. M. A. **134**:1143, 1947.

24. Tullis, J. L., and Warren, S.: J. A. M. A. **134**:1155, 1947.

cants on the dog than on the rat is also noted with respect to the spleen, the bone marrow, the small intestine, the liver and the adrenal glands. The present findings indicate that not only are the vesicants rapidly acting specific poisons, but they have a slower, more general intoxicating effect. This effect seems to be associated with damage of the capillaries, since in the lymph nodes, the spleen and the adrenal glands there is evidence of sloughing of the endothelium, congestion and hemorrhage. These pathologic changes occurring in the capillaries are particularly damaging to organs with rich blood supply, and it is possible that they caused the cystic degeneration found in the kidney of one of the dogs repeatedly poisoned with HN-3. The secondary pathologic changes, while not influencing the blood picture directly, may produce conditions in the lymph nodes and the spleen which prevent adequate transport of such materials as may be absorbed from the damaged small intestine and which are necessary for the adequate metabolism of the whole animal.

With sublethal doses the damage produced by the vesicants is largely on the proliferative cells, and judging from the regenerative changes in the blood, one concludes that the injured hemopoietic centers can regenerate. Myeloid cells start to regenerate within eight or nine days after the initial poisoning, and the regeneration is indicated by a shift to the left of the neutrophilic granulocytes of the blood, but the lymphocytes, although showing slight regenerative capacities during the second week, never reach such relatively high values as do the neutrophils. The thrombocytes follow about the same pattern of regeneration as do the neutrophils. With lethal doses the damage to both the proliferative cells and to the organ structure, particularly the capillaries, is such that regeneration is slow or does not take place. Extensive pathologic changes in the lymphoid organs are often accompanied by bacterial invasion and necrosis of tissue not damaged by the agents. Such bacterial invasion has been seen in dogs in which normal flowing of lymph through the lymph nodes has been blocked.[25] Furthermore, the vesicants have been shown to prevent antibody formation,[26] the absence of which may account for the observed bacterial invasion of the lymphoid organs of the poisoned dogs.

Another reaction which has been observed in the poisoned dogs and which remains unexplained is the relative hyperplasia of the plasma cells in the bone marrow at the time when the myeloid and erythroid cells have decreased in number. The plasma cells appear to be derived from lymphocytes and not from reticulum cells as they are in the bone

25. Drinker, C. K.; Field, M. E.; Ward, H. K., and Lyons, C.: Am. J. Physiol. **112**:74, 1935.

26. Spurr, C. L.: Proc. Soc. Exper. Biol. & Med. **67**:259, 1948.

marrow of rabbits after anaphylactic shock.[27] Plasma cell hyperplasia has also been found in the hypoplastic bone marrow of the victims of atomic bomb radiation.[23] Regardless of their origin, the plasmacytes are always present in the hypoplastic marrow and seem to be formed in greater numbers when there is no accumulation of degenerated cells.

Aside from the secondary pathologic effect of the vesicants on the hemopoietic organs, the pathologic changes observed in the adrenal glands could partially account for the somatic and visceral conditions of general debility, muscular weakness, etc., present in the poisoned dogs at death, since both the symptomatic conditions and the pathologic changes of the adrenal glands resemble those of dogs in which the veins of the adrenal glands have been ligated.[17]

The observations made in this report of the degenerative changes in the nerve cells of the autonomic ganglions in the poisoned dogs lend anatomic support to the pharmacologic observations that one of the cyclic derivatives produced by the hydrolysis of the 2-chloroethyl vesicants has a paralytic effect.[28] If great numbers of nerve cells should be damaged, it would appear that the abnormal physiologic conditions observed in the alimentary systems of the poisoned dogs could be attributed to such damage.

Unfortunately, at the present time there does not seem to be an agreement as to how the initial nucleotoxic effect of the 2-chloroethyl vesicants is produced.[29] Selective physical damage is done to the cells which react with tagged phosphorus,[30] to cells that contain high concentrations of desoxyribose nucleic acid,[31] to cells whose membranes are made more permeable by roentgen rays in vitro[32] and to cells which contain a physically demonstrable content of an alkaline phosphatase in the nucleus.[33] Poisoning could occur directly through the blocking of the enzyme systems necessary for the high metabolic activity incident to mitosis. The various possibilities for selective reaction have been discussed at length by Gilman and Philips,[29] but the explanation of the reaction is still in doubt.

SUMMARY

Daily counts of white and red blood corpuscles and thrombocytes in dogs poisoned with varying amounts of bis(2-chloroethyl) sulfide and the hydrochlorides of ethyl-bis(2-chloroethyl) amine and tris(2-chloro-

27. Good, R. A.: Proc. Soc. Exper. Biol. & Med. **67**:203, 1948.
28. Philips, F. S., and Gilman, A., in Moulton,[2] p. 3.
29. Philips and Gilman.[28] Gilman and Philips.[21]
30. Andreasen, E., and Ottensen, J.: Acta path. et microbiol. Scandinav., 1944, supp. 54, p. 25.
31. Gjessing, E. C.: Federation Proc. **7**:156, 1948.
32. Schrek, R.: J. Cell. & Comp. Physiol. **28**:277, 1946.
33. Wislocki, G. B., and Dempsey, E. W.: Anat. Rec. **96**:249, 1946.

ethyl) amine showed significant relations between the neutropenia, lymphopenia, anemia and thrombopenia observed in the peripheral blood and the hypoplasia of cells and inhibition of mitosis observed in the lymph nodes, the spleen and the bone marrow.

Secondary pathologic changes were present in all of these organs, and these are believed to have interfered with the proper functioning of these organs and to have imposed conditions which prevented normal regeneration. In addition, pathologic changes were present in the adrenal glands, small intestine, cecum, colon, liver, pancreas, kidneys and tonsils which are thought to have been caused by the damage done to the capillaries and which contributed to the general conditions of intoxication which occurred in these dogs.

The hemopoietic organs of the dog seem to be more susceptible to the damage caused by the 2-chloroethyl vesicants than are those of the rat.

EFFECT OF ANTI-RAT-LIVER SERUM ON RATS

HUBERT R. ESTES, M.D.
CHICAGO

THE ROLE of specific tissue sensitivity as a factor in the genesis of disease has been investigated by many workers. Most of their experiments have involved either the administration of an antigen to which the animal has previously been made sensitive or the administration of antibodies specific for certain tissues of the animal. In regard to this second group it has been hypothesized that the animal's own tissue acts as an antigen, reacting with the artificially provided antibody.

HISTORY

Lindemann,[1] in 1900, while investigating the effect of various chemical and biologic poisons on the kidney, produced an antiserum specific for rabbit kidney by immunizing guinea pigs against the renal substance of rabbits. By administration of this specific antirenal serum to rabbits, he succeeded in producing severe tubular degeneration and necrosis. The glomeruli showed no changes except for small amounts of albumin in an occasional capsular space.

This work was confirmed by Pearce[2] in 1903. However, he showed that some of the effects observed previously were not due to antibodies specific for renal tissue but were due to antibodies specific for serum and red blood cells. To remove this variable factor, he introduced the procedure of perfusing the organs to be used as antigen with saline solution before they were removed and ground up.

In 1933 Masugi[3] succeeded in producing glomerulonephritis in rabbits by the use of nephrotoxic serum. In his original experiments rabbits were given repeated injections of a suspension of rat kidney, and their serum was subsequently injected into rats. Later he immunized ducks against rabbit kidney and then administered this antirenal serum to rabbits. On examination of the kidneys of these animals, he found proliferation of the glomerular endothelium, edema of the capillary walls, fibrinoid masses and hyalinization within the glomerular tuft, capsular exudate consisting of albumin, red blood cells and desquamated epithelium, adhesions between tuft and capsule, crescent formation and interstitial fibrosis with cellular infiltration.

From the Department of Pathology, Northwestern University Medical School.

1. Lindemann, W.: Ann. Inst. Pasteur **14**:49, 1900.
2. Pearce, R. M.: Univ. Pennsylvania M. Bull. **16**:217, 1904.
3. Masugi, M.: Beitr. z. path. Anat. u. z. allg. Path. **92**:429, 1934.

The work of Masugi was confirmed and extended by Smadel,[4] Smadel and Farr[5] and Swift and Smadel.[6] These authors reviewed the literature on renal lesions produced by the use of specific antirenal serum and reported that glomerulonephritis had been produced in rats by the use of such serum. They showed that by an adjustment of the dose of serum the chronic stage of the disease could be reproduced. Their conclusions were supported by clinical, functional and pathologic studies of the test animals. The authors differentiated between lesions caused by specific antirenal serum and those caused by serum containing antibodies specific for serum and blood cells, and emphasized the importance of removing all blood by perfusion from the organ to be used as antigen, as first advocated by Pearce. Recently, Seegal and Loeb[7] have shown that glomerulonephritis can be produced in rats by the injection of specific antiplacental serum. In addition, these authors showed that the "in vitro" titration of antiserum does not necessarily correspond to its activity in vivo.

In addition to administering specific antirenal serum, Masugi[8] also administered specific antihepatic serum to rats. Rabbits were immunized with a suspension of rat liver to provide the specific antiserum. He removed antibodies specific for the red blood cells and the serum of rats by mixing the rabbit serum with rat red blood cells and serum. This, he believed, would remove any antibodies to red blood cells or to serum by absorption. Subsequent serologic tests showed the precipitin titer of the mixed serums to be 0 for rat red blood cells, 80 for rat serum and 320 for rat liver. Twenty-four to forty-eight hours after injection of the mixed serum, autopsy showed hyaline changes, fatty metamorphosis and necrosis of liver cells, particularly in the peripheral and intermediate zones of the hepatic lobules. There was also cellular infiltration in which neutrophilic granulocytes, lymphocytes and many eosinophilic granulocytes participated.

In an attempt to produce cardiac lesions similar to those found in rheumatic fever, Bauer[9] prepared serum specific for rat heart by administering a suspension of rat heart to rabbits in successively larger doses. The rabbit serum showed precipitins specific for rat heart in a dilution of 1:4,000. There were also variable smaller amounts of antibody to rat blood serum as well. Rats receiving large doses (0.3 to 2 cc.) of this antiserum into the femoral vein died within a few minutes to one-half hour with the clinical picture of anaphylactic shock. Autopsy

4. Smadel, J. E.: J. Exper. Med. **64**:921, 1936; **65**:541, 1937.
5. Smadel, J. E., and Farr, L. E.: J. Exper. Med. **65**:527, 1937.
6. Swift, H. F., and Smadel, J. E.: J. Exper. Med. **65**:557, 1937.
7. Seegal, B. C., and Loeb, E. N.: J. Exper. Med. **84**:211, 1946.
8. Masugi, M.: Beitr. z. path. Anat. u. z. allg. Path. **91**:82, 1933.
9. Bauer, F. C.: Arch. Path. **42**:222, 1946.

showed severe hyperemia, edema and hemorrhage of the lungs. Other organs showed only slight hyperemia. Smaller doses over a period of one to three weeks caused lesions in the lungs, which consisted of thickening of the alveolar walls with fibrinoid material and connective tissue cells. Rats receiving intraperitoneal injections showed marked fibrosis of the parietal and visceral peritoneum. The authors pointed out that the serum contained specific antibodies for all the tissues found in the heart, that is, cardiac muscle, connective tissue, endothelium and blood serum, and therefore the first of these tissues encountered in the recipient's body would react with the antibody and fix it. This was thought to explain the marked reactions of the lungs when the serum was injected intravenously and the peritonitis when it was injected intraperitoneally.[10] There were no significant findings in the hearts.

In consideration of these attempts to produce lesions in an organ by the administration of antiserum specific for the tissue of that organ, the question arose whether or not lesions might be produced in the liver by the administration of antihepatic serum. Masugi reported that relatively large doses of antihepatic serum produced microscopic lesions in the liver within twenty-four to forty-eight hours. The present study was undertaken to determine the effect of smaller doses of antihepatic serum administered over a period of months, as well as the effect of large doses administered for a few days.

MATERIAL AND METHODS

Preparation of Specific Anti-Rat-Liver Serum.—White rats were killed with ether, and the livers were removed and perfused with isotonic solution of sodium chloride until the return fluid was clear. They were then chopped into small pieces and washed in continuously running tap water for twelve to eighteen hours. Then the pieces were placed in a mortar and pestle and ground until a smooth paste was prepared. It was found that sand was not necessary for grinding. The paste was diluted with isotonic solution of sodium chloride to make a 10 per cent suspension and merthiolate® was added to make a 1:5,000 concentration. After twenty-four hours this antigen suspension showed no growth on dextrose agar.

This liver suspension was administered intraperitoneally to 4 rabbits at three day intervals. The dose schedule is recorded in the table. No untoward reactions occurred. One week after the last injection was given, 10 cc. of blood was taken from the ear vein of each rabbit to determine the antibody titer. This was done by the precipitin test recommended by Zinsser and Bayne-Jones.[11] Precipitation occurred in the serums of 3 rabbits in a dilution of 1:2,000 after one hour at room temperature and in that of the fourth rabbit in a dilution of 1:500. This rabbit was not used. Precipitation also occurred with rat serum (1:1,000 after one hour) and rat kidney (1:100 after two hours). There was no precipitation with rat heart.

10. Stenn, F.: Arch. Path. **26**:244, 1938.

11. Zinsser, H., and Bayne-Jones, S.: A Textbook of Bacteriology, New York, D. Appleton-Century Company, Inc., 1939, p. 932.

The 3 rabbits showing high titers were bled from the ear vein, about 50 cc. of blood being taken from each. The serum was separated from the blood and pooled, and merthiolate® was added to make a 1 : 5,000 dilution. After twenty-four hours there was no growth on dextrose agar.

Administration of Anti-Rat-Liver Serum to White Rats.—The animals were divided into two groups. Group A received large doses for a short period (2 cc. every other day for two days, five days and two weeks), and group B received small doses for a long period (0.3 cc. every other day for three months). Each group was divided into three series. Series P received the liver antiserum; series N received normal rabbit serum; series A received a 5 per cent solution of egg albumin. All injections were intraperitoneal. The doses were the same for experimental and control animals in each group. Three other rats were maintained on the same diet as the experimental animals as food controls. All animals were fed a commercial dog chow[12] and allowed as much water as they would drink. Their weights varied from 166 to 304 Gm. They were from a mixed laboratory strain, and the series included both males and females.

Schedule for Administration of Rat Liver Antigen to Rabbits

Days	Cc. of 10% Rat Liver Antigen
1	1
4	2
7	3
10	4
13	6
16	9

The rats were killed with ether, and the autopsy material was fixed in buffered 4 per cent formaldehyde solution, Zenker's solution and 70 per cent alcohol. Sections were stained by the hematoxylin-eosin method, Mallory's technic for connective tissue, Mallory's phloxine technic, the silver reticulum method, Best's carmine technic for glycogen and with periodic acid.

RESULTS

Within a few seconds after injection of the anti-rat-liver serum, the animals acted as though in extreme pain. Their heads drooped, their eyelids fell and finally the rats rolled on their sides and backs. They were quite restless and changed positions frequently. All rats seemed to recover after about five minutes. The severity of the reaction decreased with each injection, until, after about eight or ten injections, the rats no longer exhibited such signs following the administration of this serum. Those receiving albumin showed no reaction.

At autopsy there were no abnormal gross findings in any rats. The peritoneum was inspected for signs of peritonitis, but there were none. All organs were grossly normal. Of the 6 rats in group A, series P (large doses of antihepatic serum for short periods), the 2 animals that had been killed forty-eight hours after a single dose of 2 cc. of the liver antiserum and the 2 that had been killed after four days of injections every other day showed scattered small granulomatous lesions, consisting of areas of necrosis of liver cells with infiltration of mononuclear cells, giant cells and a few lymphocytes and eosinophilic granulocytes. In addition there

12. Wayne Dog Blox® food pellets.

were occasional single giant cells scattered throughout the hepatic parenchyma. An occasional liver cell was swollen and filled with hyaline material, and its nucleus was fragmented. The kidneys, spleen, intestine and lungs were all histologically normal. The 2 animals in this group that received an injection every other day for two weeks did not show any lesions. The liver and other organs were histologically normal. The control animals (those that received normal serum or egg albumin and the food controls) did not have any histologic lesions in the liver or other organs. No histologic lesions were observed in the liver, the kidney or other organs in either the experimental or the control animals of group B. Best's carmine stain showed a slightly decreased amount of glycogen in the livers of all the animals, but there was no difference in glycogen content between the experimental and the food control animals.

COMMENT AND SUMMARY

In these experiments an anti-rat-liver serum was administered intraperitoneally to white rats for periods of two days, four days, two weeks and three months. The serum was prepared by injecting a suspension of ground perfused and washed rat livers into rabbits, and it had an anti-rat-liver titer of 1 : 2,000.

Scattered small granulomatous areas of focal necrosis and cellular infiltration were observed in the livers of the rats that had received one 2 cc. dose, with autopsy forty-eight hours later, and in the rats that had received two 2 cc. doses over four days, with autopsy forty-eight hours after the last dose. The rats that had received 2 cc. of liver antiserum every other day for two weeks and those that had received 0.3 cc. every other day for three months, as well as all control animals to which normal rabbit serum or 5 per cent egg albumin had been administered, suffered no lesions in the livers, kidneys, lungs, peritoneum or other organs that could be detected with the naked eye or by ordinary histologic examination.

It is well to note here that Seegal and Loeb [7] have shown that the toxicity of an antiserum is not always directly proportional to its specific antibody titer.

The areas of focal necrosis occurring in the liver after only one or two injections of antihepatic serum is in agreement with the works of Masugi.[8] However, after the administration of this serum had been continued for weeks or months, it did not cause any lesions in the liver or other organs. One explanation for this observation may be the hypothesis that the liver was damaged by the initial dose of antiserum but quickly recovered and developed resistance or tolerance to subsequent doses.

SIGNIFICANCE OF DUCTAL SCLEROSIS IN PAGET'S DISEASE

Regression of Intraductal Carcinoma

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THAT carcinoma of the mammary ducts has been associated with Paget's disease of the nipple in most, if not all, of the cases studied is generally recognized. Haagensen [1] stated, "In Paget's erosion of the nipple meticulous microscopic search of the breast will always reveal somewhere in it a primary carcinoma whose cells have grown along the ducts to reach the surface of the nipple." However, there are instances in which a ductal carcinoma has not been found despite typical changes of Paget's disease in the nipple [2]; in some of the patients carcinoma was discovered subsequently in the regional nodes or elsewhere, indicating that it must have been present in the first place. In such cases it has generally been assumed that the carcinoma of the ducts must have been overlooked. Study of a recently observed case of Paget's disease of the nipple, in which ductal carcinoma was found only with difficulty and in which evidence of considerable regressive change was noted, has suggested another explanation of this puzzling phenomenon. Analysis of this case provides a clue to an understanding of those instances in which carcinoma of the ducts is apparently not present.

A 54 year old white woman had noted a small red area on the left nipple for seven weeks. This area burned and itched and did not respond to local applications. The nipple presented a small granular eroded area; biopsy resulted in a diagnosis of Paget's disease of the nipple (carcinoma simplex of the nipple). Mastectomy and axillary dissection were done.

Examination of the specimen (by Dr. Nathan B. Friedman) showed that the breast measured 16 cm. in greatest diameter and that the covering ellipse of skin was 15 by 6 cm. and contained an everted nipple, the surface of which was roughened and eroded. Microscopically, the skin of the nipple had isolated Paget's cells scattered about in the epidermis and occasionally larger aggregations of similar elements, which formed nests bulging into the underlying dermis similar to those seen in junctional nevi. An occasional cleavage plane had formed just above the basal layers of the epithelium. There was a moderate degree of inflammatory cellular infiltration along the dermoepidermal border.

From Cedars of Lebanon Hospital.

1. Haagensen, C. D.: J. A. M. A. **138**:195, 1948.

2. Geschickter, C. F.: Diseases of the Breast, Philadelphia, J. B. Lippincott Company, 1945.

Sections through the main ducts were not remarkable, nor were sections from many other portions of the breast. In view of the absence of microscopic changes in the mammary tissue itself, the entire gross specimen was subjected to meticulous

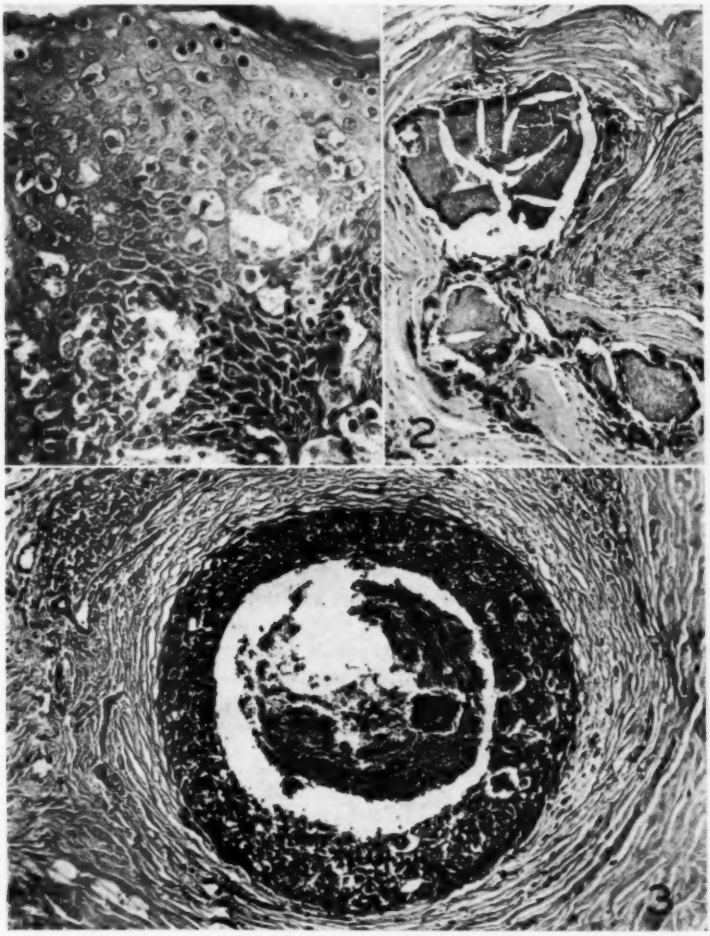

Fig. 1.—Microscopic view of nipple. The epidermis shows many swollen and bizarre Paget cells.
Fig. 2.—Sclerosis and calcification of obliterated ducts.
Fig. 3.—Intraductal carcinoma with periductal sclerosis.

reexamination. This resulted in the disclosure of a small focus, slightly scarred, about 1 cm. in diameter, located about 5 cm. from the nipple. Tiny, gritty granules

were embedded in this focus. Microscopically, the scarred area revealed many masses of necrotic and calcified material, which was embedded in and surrounded by lamellated collagen suggesting preexisting tubular structures. Giant cells and macrophages were present next to some of the concretions. In other sections, where there was frank intraductal carcinoma, viable neoplastic tissue could be recognized in the lumens of several ducts in close juxtaposition to the necrotic and calcifying debris.

COMMENT

Cheatle and Cutler [3] described similar findings in Paget's disease and emphasized the hyperplasia of the subepithelial connective tissue of the ducts with considerable proliferation of elastic fibers They interpreted this as possible evidence of regression of preexisting neoplasm in some areas. It is not possible on the basis of the present case to state whether complete regression of the ductal carcinoma can occur, although this is a possibility which should be looked for in the study of other cases. If such regression can be complete, the scarred ducts might be ignored during the search for carcinoma of the ducts, since it would not be realized that this scarring represented previous carcinoma. Such a combination of observations might account for cases in which no carcinoma of ducts has been found and even for those in which subsequent carcinomatous involvement of lymph nodes or a scar of the skin was disclosed.

SUMMARY

A case of Paget's disease of the nipple in which there was an underlying ductal carcinoma of the breast is described. The carcinoma was found only after the gross specimen had been scrutinized minutely. There was evidence of considerable regression of the neoplastic process with resultant sclerosis and calcification of the involved ducts. The finding of sclerosis of ducts in Paget's disease should be taken as possible evidence of preexistent carcinoma. This observation might explain those cases of Paget's disease in which carcinoma of the ducts has not been found.

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3. Cheatle, G. L., and Cutler, M.: Tumors of the Breast, Philadelphia, J. B. Lippincott Company, 1931.

Laboratory Methods and Technical Notes

TISSUE CULTURE STAINING "IN SITU"

MACHTELD E. SANO, M.D.
AND
CAROL A. BOCHER, B.S.
PHILADELPHIA

SINCE 1930 when one of us (M.E.S.) with Dr. Lawrence W. Smith [1] developed a simplified method of tissue culture, attempts to fix and stain the latter "in situ" have met with little success. Removing the clot from the large cover slip after fixation and subsequent embedding produced too much distortion for the study of cytoplasmic inclusions and nuclear detail. Our main difficulty was shrinkage and turbidity of clot with resultant poor clearing. Since the advent of Earle's [2] method both these obstacles have been overcome. If the stains we use are different from those of Dr. Earle it is not that we find his unsatisfactory but that for our particular purpose, i. e., a study of human tumors, a more contrasting stain is desirable. The underlying principle of avoiding shrinkage by using graded mixtures of alcohol is identical. Our method is a combination of Earle's fixation and dehydration procedures and Masson's trichrome stain as modified by Pollak.[3]

METHOD

The cover slips used in the present method are 62 by 62 by 1 mm., and the diameter of the rings is 28 mm. Five to six or more fragments of tissue are implanted in this ring and grown in 6 drops of medium. This medium varies in quality according to the cultures, but the total quantity always remains the same. The thickness of the clot is such that cellular detail is perceptible in different depths of the medium.

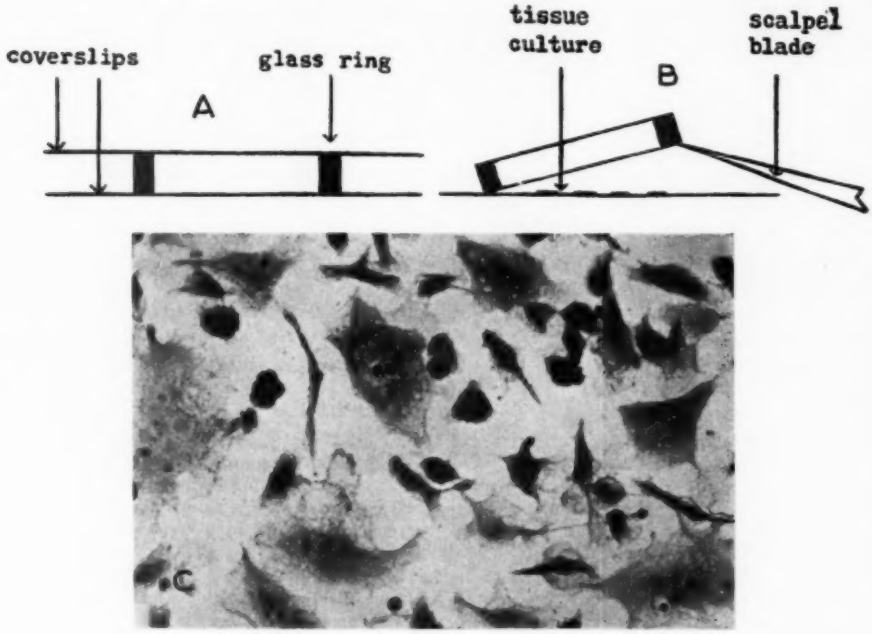
For the detailed preparation of solutions we refer to the original articles. All solutions, including formaldehyde and potassium dichromate solutions, must be filtered in order that cultures may be kept free from dirt particles, which adhere to the clot and cannot be washed off. City water (Philadelphia's) must be passed through ordinary filter paper (Fisher Scientific Company no. 9-795 semicrimped rapid). Filtration of solutions is exceedingly important and cannot be overemphasized.

Ten parts of 37 per cent formaldehyde solution are added to 100 parts of 3 per cent potassium dichromate solution immediately before using and filtered. The

From the Agnes Barr Chase Cancer Research Foundation Temple University Medical School and Hospital.

1. Sano, M. E., and Smith, L. W.: Proc. Soc. Exper. Biol. & Med. **28**:282, 1930.
2. Earle, W. R.: J. Nat. Cancer Inst. **8**:83, 1947.
3. Pollak, O. J.: Arch. Path. **37**:294, 1944.

solution is warmed to 38 C. in the incubator in a closed staining dish. The tissue culture to be stained is not removed from its oven until the fixing solution has attained the right temperature. To remove the top cover slip and ring from the tissue culture chamber a warmed Bard-Parker scalpel is passed over the top cover slip to melt the paraffin–petrolatum U. S. P. seal. The cover slip then slides off easily. The ring is pried loose with the warm scalpel from the bottom cover slip on which the culture has grown. Any accumulated liquid is allowed to drain off the culture. Gently immerse the cover slip in the warmed formaldehyde-dichromate mixture, making certain the cover slip stands on end and does not lie flat on the bottom of the dish. Place in the incubator at 38 C. for fifteen minutes. Refresh the



A, tissue culture chamber. In *B* the top cover slip has been removed. The ring is pried loose from the tissue culture cover slip. *C*, tissue culture of centrifuged cells from a tuberculous pleural effusion. Note giant cells, lymphocytes and fibroblasts.

fixative. Allow to stand overnight in freshly prepared fixative at room temperature. Change the fixative next day if the solution darkens. After twenty-four hours remove the cover slip from the fixing solution, avoiding the use of metals, and rinse in distilled water. Immediately place the cover slip in filtered 3 per cent potassium dichromate solution (without formaldehyde solution) for twenty-four hours. The culture can remain in this solution for seventy-two hours if necessary without the appearance of the cells being changed. The cover slip is now washed for twenty-four hours in filtered running tap water, care being taken that the cover

slip stands on end. After twenty-four hours the culture is placed in alcohol (1 part), glycerin (1 part), distilled water (2 parts) mixture. Best results are obtained when it is left in this mixture overnight. Again, if it is left seventy-two hours in this solution, no deleterious effects are noted.

The fixed tissue culture is now ready for staining. It is placed in Harris' hematoxylin for two minutes (as in all staining procedures the period may vary according to the individual batch of stain). Drain the staining solution off and wash for twenty minutes in filtered running tap water. Stain in Pollak's [3] trichrome solution for ten to fifteen minutes. Differentiate shortly in acidified distilled water (0.2 per cent acetic acid).

Dehydrate through 35, 50, 70, 80, 95 per cent alcohol and then absolute alcohol, three minutes in each solution. Clear in Earle's series of six solutions, absolute alcohol, acetone and toluene mixtures, five minutes in each solution. Mount in clarite® dissolved in toluene of a 3 by 2 inch (7.6 by 5 cm.) slide of standard thickness.

COMMENT

Like tissue culture itself, the method is not difficult to master once the necessary steps have been well established. The tissue culture method referred to [1] permits the study of the cells under high power and under oil immersion if desired. Later, the modified staining method described in the foregoing section of this article permits the study of these very same cells after fixation. The method has an advantage over the regular hanging drop method in permitting simultaneous studies of numerous fragments. It is inexpensive and simple, and it allows for endless variation of experiments.

Books Received

THE RENAL ORIGIN OF HYPERTENSION. By Harry Goldblatt, M.D., C.M., director, Institute for Medical Research, Cedars of Lebanon Hospital, and professor of pathology, University of Southern California, Los Angeles. Publication no. 14, American Lecture Series: A Monograph in American Lectures in Pathology. Pp. 126. Price $2.75. Springfield, Ill.: Charles C Thomas, Publisher, 1948.

This is an excellent summary of present day knowledge concerning the renal origin of hypertension. Written by one of the leading specialists, every chapter bears the imprint of intimate familiarity with the subject, gained in many years of research.

Although the work of others is also considered, the volume is mainly based on the author's investigations. This would be a disadvantage in the case of most monographs, but Goldblatt has done so much to increase knowledge in the field of renal hypertension that this manner of presentation actually adds to the value of the book.

It is perhaps somewhat inconvenient that the bibliographic list includes only six entries. However, as the author suggests, these key references will help the reader to find additional pertinent original publications. This system may be somewhat inconvenient, but as the book was primarily written for those not specializing in research on hypertension, it will not be felt as a great drawback.

It would be difficult to give more information concerning the well established aspects of this vast subject in the space of 126 pages. The volume is attractively bound; illustrations, composition and paper all reach that high degree of excellence to which readers have been accustomed in Charles C Thomas books. The volume is highly recommended to all those interested in hypertension.

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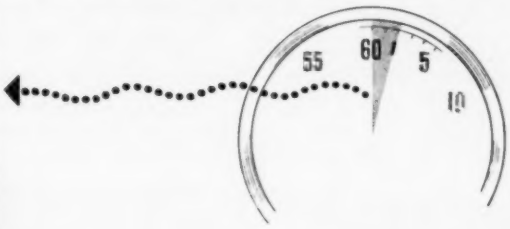

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